ISSN No: 0976-5921

Research Article

Deciphering the Neuroprotective Role of Ksheerabala: Bridging Modern Medicine and Ayurveda Through Reverse Pharmacology

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Received: 25-03-2025 Accepted: 26-09-2025 Published: 30-09-2025

Abstract

Ksheerabala (KB), a traditional Ayurvedic formulation, is widely recognized for its therapeutic potential in managing various neurological and musculoskeletal conditions, including cervical spondylosis, cerebral palsy, facial paralysis, low back pain, sciatica, and osteoarthritis. Noted for its anti-inflammatory and neuroprotective properties, KB is examined in this study through a reverse pharmacology approach that bridges ancient Ayurvedic wisdom with modern scientific validation. The study evaluated KB's efficacy by assessing its antioxidant activity using DPPH assay, ferric reducing power assay, and superoxide scavenging assay. Additionally, phospholipase A^2 (PLA^2) inhibition and calcium channel modulation were investigated. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed to identify its bioactive constituents. *In vitro* experiments demonstrated significant suppression of PLA^2 activity, with an IC_{50} value of 28.58 µg/mL, alongside potent free radical scavenging effects confirmed by the antioxidant assays—highlighting KB's anti-inflammatory potential. Furthermore, KB reduced voltage-gated calcium channel activity, as evidenced by a significant, dose-dependent down-regulation of Cacna1b gene expression across concentrations ranging from 15 to 60 µg/mL. The GC-MS analysis identified multiple bioactive compounds that likely contribute to KB's analgesic and neuroprotective effects. Overall, the findings support KB as a promising natural therapeutic agent for neuropathic pain. By integrating traditional medicine with modern pharmacological techniques, this study provides a scientific foundation for future preclinical and clinical research on KB's efficacy and safety.

Keywords: Calcium channel regulation, Phospholipase A^2 inhibition, Neuroprotection, Neuropathy pain, *Ksheerabala*

Access this article online

Website: https://ijam.co.in

DOI: https://doi.org/10.47552/ijam.v16i3.5970

Introduction

Neuropathic pain (NP), as defined by the International Association for the Study of Pain, results from lesions or dysfunction within the somatosensory system and is characteristically chronic, debilitating, and resistant to standard therapies (1). It commonly arises from conditions like diabetes, cancer, alcoholism, spinal cord injury, and chemotherapeutic neurotoxicity (2,3,4). The pathophysiology of NP is complex and multi factorial, involving oxidative stress (5), ion channel dysregulation (notably N-type and T-type calcium channels)(6,7), and neuro-inflammation (5) mediated by activated microglia and inflammatory mediators like phospholipases and cytokines (8,9,10,11,12). Current pharmacological treatments—including tricyclic antidepressants, anticonvulsants, and opioids—offer limited efficacy and are associated with significant adverse effects, including cardiotoxicity and addiction risk (13,14,15). This underscores the urgent need for novel, safer, and more effective therapeutic options.

Ayurveda, one of the world's oldest holistic medical systems, offers a unique and comprehensive framework for managing chronic and complex conditions such as neuropathic pain (NP) (16,17). Increasingly acknowledged in modern pharmacological research, Ayurveda has inspired the development of reverse pharmacology—a contemporary drug discovery approach that begins with clinical observations and traditional medicinal practices rather than laboratory-based molecular targeting. This approach accelerates drug development by identifying potential therapeutic agents from traditional remedies and investigating their mechanisms of action and efficacy (18,19). Among various Ayurvedic formulations with neuroprotective potential,

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Ksheerabala (KB) is a well-known nerve tonic whose use is documented in the ancient Ayurvedic text *Ashtangahriday*a (20).

In Ayurvedic texts, *Ksheerabala* is described as *balya* (strengthening), *vedanasthapana* (analgesic), and *vatashamaka* (*vata* pacifying) These characteristics suggest its therapeutic relevance for *vata* disorders (20), which, in Ayurvedic pathology, align with conditions involving nerve dysfunction and degeneration—closely mirroring the pathophysiological constructs of NP. KB is an Ayurvedic oil formulation prepared using cow's milk (*Go-Ksheera*), *Bala* (*Sida cordifolia*), and sesame oil (*Tila Taila*). Cow's milk is rich in essential nutrients necessary for the growth and nourishment of bones, nerves, muscles, and other tissues in the body (21).*Sida cordifoli*a is a highly esteemed ayurvedic herb, extensively used in the treatment of neurological and cardiac conditions, and is known for its analgesic, anti-inflammatory, and hepatoprotective properties and contail ephedrine as major alkaloid (21,22,23,24). Sesame oil contains the crystalline compound sesamin and the phenolic compound sesamol. It is commonly used as a base oil in the preparation of various medicinal oils helps in absorption by gastrointestinal tracts. Sesame oil strengthens and nourishes all *Dhatus* (tissues), prevents their depletion (*Dhatukshaya*), and alleviates inflammation and neurological disorders (*vata*) (21). The combination of these ingredients enables KB to address a wide range of *vata*-related disorders , including conditions such as cervical spondylosis cerebral palsy facial paralysis , low back pain , sciatica, osteoarthritis, hemiplegia , parkinson's disease , convulsions, and other neurological ailments (25,26,27), improves diabetic retinopathy (28) and improves vision in Stargardt's disease(29),oral submucous fibrosis (30). Studies including those by Swathy et al., demonstrate KB's ability to mitigate neurotoxicity through its antioxidant and neuroprotective properties, affirming its role as a valuable remedy for neurological conditions(31,32). As far as we are aware, no research has looked into how KB formulations might help with neuropathic pain. This study aims to address this gap by evaluating the free radical scavenging properties, phospholipase inhibition, and calcium channel inhibition—key mechanisms underlying neuropathic pain pathophysiology—using *in vitro* models.. Additionally, the study examines the formulation's and chemical composition through GC-MS analysis, providing a scientific foundation for its potential therapeutic applications.

Materials and Methods

To assess antioxidant potential, *in vitro* assays were performed, namely DPPH radical scavenging, ferric reducing power, and superoxide (O_2^-) radical scavenging assay. The role of KB in modulating neuro-inflammation was evaluated focusing on the involvement of phospholipase enzymes. Additionally, gene expression studies were conducted on microglial cells to examine the effects of KB on calcium channels implicated in NP. Gas Chromatography–Mass Spectrometry (GC-MS) analysis was also carried out to identify the bio-active constituents present in KB**.**

Chemicals: KB was procured from the local market. Chemicals 1,1-diphenyl 2-picrylhydrazyl (DPPH), ethanol, phosphate buffer, trichloroacetic acid, riboflavin, Nitroblue tetrazolium (NBT), Edetate disodium (EDTA),Sodium chloride ,Calcium Chloride ($CaCl^2$), Dimethyl Sulphoxide (DMSO) and all other chemical reagents of analytical grade were purchased from TCI Chemicals (India) Private Limited.Egg yolk phosphatidylcholine or lecithin, red phenol and sodium taurodeoxycholate (NaTDC), were purchased from Sigma Chemical Co.

The UV spectrophotometric measurement for assessing the *invitro* antioxidant activities using different strategies carried observed using Shimadzhu 160A UV-Visible spectrophotometer.

***In vitro* Antioxidant Assays**

The antioxidant activity of KB was determined by three *in vitro* assay methods. They are DPPH assay, Ferric reducing power assay and superoxide scavenging assay

DPPH radical scavenging activity

The scavenge ability of the sustained free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), as reported by Bharati et al was used to assess the antioxidant activity of the sample (33). One milliliter of 0.1 mM DPPH in ethanol was combined with one milliliter of KB at various concentrations (20–120 µg/mL) generated in ethanol. .The reaction mixture was incubated in the dark for 30 minutes. A control sample consisted of 1 mL of ethanol mixed with DPPH solution in 1 milliliter. A UV-Visible spectrophotometer was used to detect the samples' absorbance at 517 nm, and a percentage of radical DPPH inhibition was computed in accordance with the results..

Absorbance % of DPPH radical solution = {(control - sample) ÷ (control)} × 100

Assay for ferric reducing power

0.2 M phosphate buffer (pH 6.6), 1 mL of 1% ferricyanide potassium solution, and different concentrations of KB in ethanol (20–120 µg/mL) were combined. After the mixture was incubated for 20 minutes at 50 °C, 1 milliliter of a 10 percent solution of trichloroacetic acid was added. As explained by Langley-Evans(34), 1 mL of a newly made 0.1% ferric chloride solution was then added, and the absorbance of the resultant solution was measured at 700 nm.

Absorbance %=[(Abs (sample)-Abs(control)/Abs(sample)] × 100

Superoxide (O_2)-radical scavenging activity

Superoxide scavenging activity was performed according Pooja et al with slight modification(35).The reaction mixture containing different concentration (20-120µg/mL) of test sample in ethanol was followed by the addition of 50 mM of a buffer with phosphate (pH 7.8), 1.5 mM of riboflavin, 12 mM of EDTA, and 50 mM of NBT.

The reaction was initiated by illuminating the reaction mixture for 150s and soon after the illumination, the absorbance was measured at 590 nm.

% Superoxide (O^2) radical = [(Abs(control)-Abs(sample)/ Abs(control)] ×100

Inhibition of Phospholipase A2 activity

To prepare 3.5 mM egg yolk phospholipids at pH 7.6, a solution of 0.1 M sodium chloride, 0.01 M $CaCl_2$, 3 mM NaTDC, and 0.055 mM red phenol was used. One unit of phospholipase A2 (PLA2) activity, expressed in international units, is equivalent to the release of one µmol of titratable fatty acid per minute in these conditions. After 20 minutes of incubation at room temperature, the remaining PLA^2 activity was assessed with 0.2 µg of sPLA2 mixed with various KB dosages. To monitor the hydrolysis kinetics, the optical density at 558 nm was monitored every five minutes after adding 1 mL of PLA2 substrate solution. Control tests used the same solvent levels without KB. After that, the IC^{50} values and residual $sPLA^2$ activity were calculated (36).

Cell culture conditions

The BV2 microglial cells were purchased from the National Center for Cell Science (NCCS) in Pune, India. In addition to 100 units/ml of penicillin and 100 mg/ml of streptomycin, the cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) enhanced with 10% fetal bovine serum (FBS) of Brazilian origin (catalog reference 10270106, Gibco, USA. To ensure optimal growth and viability, the culture was incubated at 37°C in a humidified environment with 5% CO_2 and 95% air.

Assay for cell viability

BV2 cells, a specialized type of microglial cells derived from the C57/BL6 mouse strain, were used to assess cell viability following treatment with KB through the MTT assay. Cells were seeded onto 96-well plates at a density of 1×10^4 cells/well. Both with and without LPS (1 µg/mL), KB was administered at dosages ranging from 2.5 to 160 µg/mL and cultured for 24 hours.

After the treatment, the medium containing the test compound was removed, and 10 µL of a 5 mg/mL MTT solution was added to each well. The cells were then incubated for an additional four hours. Subsequently, 150 µL of DMSO was added to dissolve the formazan crystals, and the mixture was left for ten minutes. The Cell viability was then assessed via measuring the intensity of absorption at 490 nm (37).

Study of calcium gene expression

BV2 microglial cells were used to perform an expression analysis of The CACNA1B gene (GenBank ID: NC_000068.8), which encodes.The voltage-gated calcium channel subunit Alpha1B was studied using mouse-specific primers. 5'-GATCCAGGAAGTCTCACCGC-3' is the forward primer. 3'-TGTGCTTTGCCCTGTTCCAA-5' is the reverse primer. To promote an inflammatory response, BV2 cells were treated with 1 µL/mL of 10% DMSO as a negative control and 1 µg/mL of LPS as a positive control.Additional groups included LPS-stimulated cells treated with different concentrations of KB (15 µg/mL, 30 µg/mL, and 60 µg/mL), with KB dissolved in DMSO. These concentrations were chosen for their significance in measuring PLA2 inhibition, allowing a dose-dependent evaluation of the compound's anti-inflammatory activities.Following a day (24hr) treatment period, RNA was extracted from the cell lysate using Trizol reagent. The PrimeScript RT MasterMix (TaKaRa, RR036A) was used to reverse-transcribe about 1 µg of total RNA into cDNA in a final volume of 20 µL.ABI7900HT was used to accomplish gene amplification with 50 ng of cDNA. A 1× TaqMan.Each reaction mixture contained Universal PCR Master Mix (Applied Biosystems), 200 nM primers, and 0.25 µL of a dual-labeled probe (Roche Probe Library). The thermal cycling conditions included an initial hold at 50°C for 2 minutes, followed by 95°C for 5 minutes for denaturation before amplification45 cycles of extension and annealing at 60°C for 1 minute and 94°C for 20 seconds.

The ΔΔCt technique and ABI Sequence Detector Software version 2.3 were used for relative quantification. Three duplicates of each measurement were created. Transcripts with a cycle threshold (Ct) value >35 were categorized as not expressed (n.e.), while those with Ct ≤35 were considered significantly expressed (38).

GC M/S Profiling of ksheerabala

KB, an Ayurvedic oil sourced from a reputable supplier in Chennai, was analyzed using GC–MS on an Agilent GC system (Model G3440A, 7890A) equipped with a 7000 Triple Quadrupole MS/MS detector. Before analysis, a 100 µL sample was properly mixed after being dissolved in 1 mL of dimethylformamide (DMF). The investigation used a DB5 MS column (30 m × 0.25 mm ID × 0.25 µm) composed of 95% methyl and 5% phenyl polysiloxane.Operating conditions included electron impact ionization at 70 eV, helium as the carrier gas at a flow rate of 1 mL/min, Temperatures for the ion source and injector are 230°C, with an auxiliary line set to 150°C.The oven temperature program began at 40°C with a 10-minute hold, followed by an increase of 5°C/min to 80°C, then 12°C/min to 220°C with a 20-minute hold, resulting in a total runtime of 32.02 minutes with a 4-minute solvent delay. Detection covered a fragment range of 45–450 Da, and Compounds were identified by analyzing their spectra and comparing them with reference data from the **NIST** and **WILEY** libraries (39).

Statistics

Results of antioxidant studies, phospholipase inhibition, and cell viability assays were expressed as Mean ± SEM, with IC_{50} values determined by linear regression. ΔΔCt data were analyzed using the Mann-Whitney test, and $p < 0.05$ was considered significant.

Results and Discussion

In vitro Antioxidant Studies

DPPH radical scavenging assay

Different concentrations (20, 40, 60, 80, 100, 120 µg/mL) of the formulation of KB were evaluated. A dose dependent percentage of inhibition of DPPH Radical was observed respectively as mentioned in Figure 1. By using linear regression analysis, ($y=0.433x+37.995$, $r^2 = 0.8461$) the IC_{50} value was determined to be 27.12 µg/mL .

Ferric reducing power assay

About 20, 40, 60, 80,100 and 120 µg/mL of the KB sample was taken for the ferric reducing power assay and the percentage of inhibition thus obtained were found to be dose dependent respectively. Based on linear regression analysis ($y=0.6445x+25.089$, $r^2 = 0.9742$) Figure 2 . The IC_{50} measurement showed that the concentration was 38.65 µg/mL.

Superoxide scavenging assay

The superoxide scavenging assay was carried out using different concentration of the

diluted KB (20, 40, 60, 80, 100 and 120 µg/mL). The percentage of inhibition was found to be 3.26, 5.61, 7.18, 18.66, 19.97 and 21.14 respectively, Figure 3.

Inhibition of Phospholipase A_2 activity

Phospholipase A2 (PLA2) activity inhibition potential of KB was assessed *in vitro* at ethanol-based doses ranging from 15 to 60 µg/mL. An IC_{50} value of 28.58 µg/mL was found by data analysis using linear regression Figure 4, ($y = 1.5454x + 5.83$, $r^2 = 0.9981$). This result suggests that KB may be useful in treating inflammatory and neuropathic pain because it effectively inhibits PLA2.

MTT Assay of BV2 cells viability

The MTT test was used to determine cell survival after exposure to KB at various dosages (2.5-160 µg/ml) with or without LPS (1 µg/mL). KB doses from 2.5 to 80 µg/mL reduced BV2 cell viability by only 18%. Even at a dosage of 160 µg/ml, BV2 cell viability remained unaffected by higher KB doses. This means

that whether KB is given alone or in conjunction with LPS, it has no significant effect on cell viability at these higher concentrations Figure 5.

Gene expression profiles induced by KB

The current work used mouse-derived microglial cells to examine Cacna1b expression in five groups. Groups 2, 3, 4, and 5 got activated by 1 µg/ml LPS, while Group 1 acted as the control group that was not activated by LPS. Stimulated groups 3, 4, and 5 received KB doses of 15 µg/ml, 30 µg/ml, and 60 µg/ml (doses studied for phospholipase inhibition). BV2 cells treated with LPS showed a 2.5-fold rise in Cacna1b expression levels, which express the α1 subunits of Cav 2.2 channels. Figure 6 demonstrates that administering KB resulted in a significant dose-dependent downregulation of Cacna1b expression in the treated groups compared to the control groups, with $p<0.05$ for the dosages employed (15-60 µg/ml).

Figure 1: Effect of KB on DPPH Radical

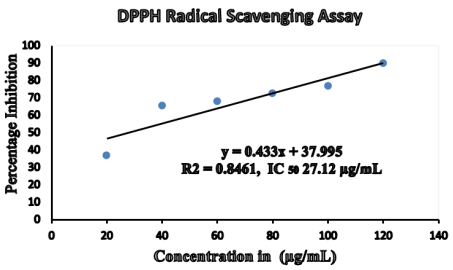


Figure 2: Effect of KB on ferric reducing power assay

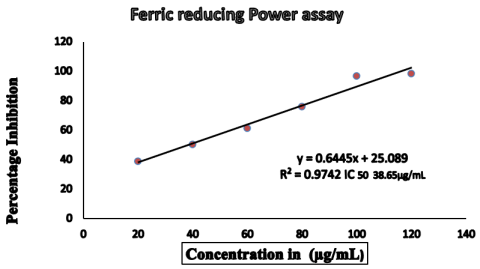


Figure 3: Effect of KB on Superoxide Radical

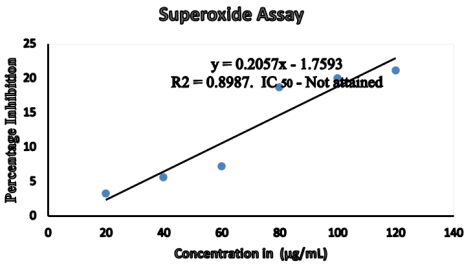


Figure 4: Effect of KB on Phospholipase A² inhibition

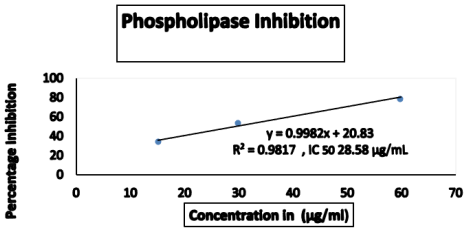


Figure 5: The effect of KB on BV2 microglia survival was assessed over 24 hours with or without 1 µg/mL LPS using MTT assays. Results, expressed as mean ± standard deviation from three independent experiments, indicate the percentage of surviving cells

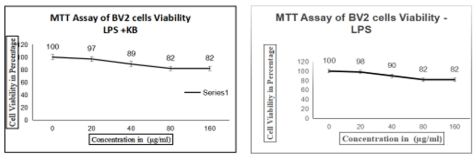
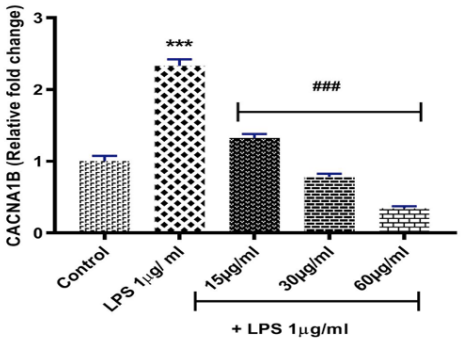


Figure 6: ΔΔCt data were conducted using the Mann-Whitney Rank Sum test, and quantitative expression data are presented as Mean± SEM. A p-value of less than 0.05 was considered significant



Each value represents the mean ± S.E.M. of three observations

GC-MS Profiling study

The GC-MS analysis of the oil revealed the presence of key components potentially responsible for KB's therapeutic effects. Based on the peak area, retention time, molecular formula, molecular weight, and percentage composition presented in Table 1 and Figure 7, the active constituents of KB were identified and confirmed as clinically relevant, particularly for chronic conditions such as neuropathic pain. The analysis highlighted the high concentrations of N-decanoic acid, dodecanoic acid (lauric acid), tetradecanoic acid (myristic acid), pentadecanoic acid, and cis-vaccenic acid, which are likely contributors to the medicinal properties of KB.

GC-M/S Chromatogram of KB

Figure 7: Indicates the GC MS graph of KB

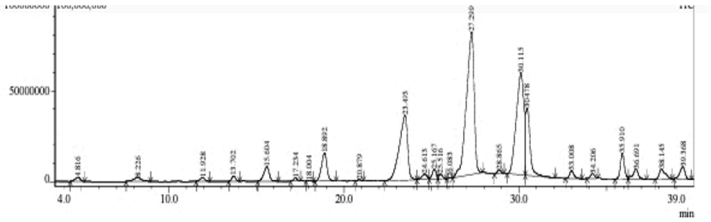


Table 1: GC–MS profile of KB showing retention time, peak area, peak height, and name of the compound

Peak#	R.Time	Area	Area%	Name
1	4.816	47653845	0.63	Butanoic acid
2	8.226	72966446	0.97	Heptanoic acid
3	11.928	37363927	0.49	Octanoic acid
4	13.702	43251180	0.57	Isopropyl butyrate
5	15.604	167301716	2.21	n-Decanoic acid
6	17.234	24427085	0.32	Propyl 2-methylvalerate
7	18.004	3806980	0.05	Benzoic acid, 4-ethoxy-, ethyl ester
8	18.892	326899980	4.32	Dodecanoic acid
9	20.879	9576875	0.13	Butyl caprylate
10	23.493	1167019082	15.44	Tetradecanoic acid
11	24.613	64659213	0.86	Pentadecanoic acid
12	25.167	89238547	1.18	Pentadecanoic acid
13	25.516	40794185	0.54	Decanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl este
14	26.083	5086117	0.07	2H-Pyran-2-one, tetrahydro-6-undecyl
15	27.299	2384606322	31.55	Pentadecanoic acid
16	28.865	30279221	0.40	Dodecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
17	30.115	1698148022	22.46	cis-Vaccenic acid
18	30.478	645713716	8.54	Octadecanoic acid
19	33.008	72157004	0.95	Tetradecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
20	34.206	39723694	0.53	2-Methyltetracosane
21	35.910	218916278	2.90	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
22	36.691	115008700	1.52	Sulfurous acid, pentadecyl pentyl ester
23	38.145	121490260	1.61	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester
24	39.368	133204323	1.76	Decyl pentyl ether
		7559292718	100.0	

Reverse pharmacology is an innovative approach to drug discovery that begins with traditional knowledge—such as the use of medicinal plants—and employs modern scientific methodologies to validate their therapeutic mechanisms (18). This process is particularly significant in the context of traditional Ayurvedic formulations like KB, which have been used for centuries to manage pain, inflammation, and neurological disorders. By leveraging reverse pharmacology, researchers can harness the empirical wisdom of traditional medicine and apply advanced scientific tools to explore the pharmacological properties of these remedies, validate their efficacy, and uncover the molecular mechanisms underlying their therapeutic effects (18).The management of neuropathic pain presents a significant clinical challenge due to its multifaceted pathophysiology, involving oxidative stress, inflammation, and neuronal dysfunction. In Ayurvedic tradition, nervous system disorders—referred to as *Vata Vyadhi*—are believed to result from imbalances in *Vata,* the biological air humor that governs both voluntary and involuntary functions. *Vata* is thought to move through the body like wind, a concept that parallels the modern understanding of nerve impulses. Peripheral neuropathy is perceived as a *Vata* imbalance, where aggravated *Vata* destabilizes the nervous system, leading to hypersensitivity, pain, and tenderness.This imbalance is often attributed to the accumulation of *Ama* (toxins) in the nervous system, resulting in nerve degeneration. In modern neurobiology, *Ama* may be correlated with the end products of oxidative stress and free radical damage(40). Another important contributor to *Vata* imbalance is the increased activity of *Srotas* (body channels), which may further exacerbate neurological dysfunction(41)

In line with this understanding, several Ayurvedic and plant-based drugs have shown beneficial effects in managing painful neuropathy. For instance, *Aconiti tuber, Alstonia scholaris,* and *Phyllanthus emblica* possess anti-nociceptive, neuro-protective, and potent antioxidant properties. Similarly, *Ginkgo biloba* has been reported to relieve neuropathic pain through its antioxidative and calcium channel inhibitory actions (42). These therapeutic effects are primarily attributed to their ability to reduce oxidative stress, protect neural tissues, and modulate calcium channels—ultimately decreasing neuronal excitability and pain perception.

Current research on KB has revealed its promising pharmacological potential in addressing these mechanisms. The *in vitro* findings demonstrate that KB can reduce oxidative damage, inhibit inflammatory pathways, and protect neuronal cells through mechanisms such as blocking ionic channels. These effects collectively underline its potential as a therapeutic agent for ameliorating neuropathic pain.

The *in vitro* antioxidant potential of KB was evaluated using DPPH radical scavenging, Ferric Reducing Power, and Superoxide Scavenging assays. KB showed potent antioxidant activity in the DPPH assay, with 89.37% inhibition at 120 µg/mL and an IC^{50} of 27.12 µg/mL, and strong ferric reducing capability, achieving 98.21% inhibition with an IC^{50} of 38.65 µg/mL. However, its superoxide scavenging activity was relatively weak, with only 21.14% inhibition at the highest concentration. These results are in line with earlier findings, where KB ameliorated quinolinic acid-induced oxidative stress (32) and attenuated alcohol-induced oxidative damage while downregulating NFκB in rat brains (31).

The antioxidative properties of KB can be attributed to its key constituents: *Sida cordifolia,* shown to outperform other Ayurvedic neurotonic herbs in antioxidant activity (43,44) milk caseins, which exhibit peroxyl and superoxide radical-trapping capacities (45) and sesame oil, known to modulate oxidative stress and enhance antioxidant defenses against Fe-induced damage (46). These constituents collectively contribute to KB's neuroprotective effects, though its scavenging activity .

Growing evidence underscores inflammation as a key factor in central nervous system (CNS) disorders, including neurodegenerative diseases. The phospholipase A^2 (PLA^2) inhibition assay revealed that KB significantly inhibits PLA^2 activity (IC^{50} = 29.22 µg/mL). PLA^2 facilitates the release of arachidonic acid, a precursor of pro-inflammatory mediators like prostaglandins and leukotrienes, which exacerbate inflammation and neuropathic pain. By inhibiting PLA^2, KB may reduce the production of these mediators, alleviating pain and inflammation. Similar effects have been observed with *Triphala,* an Ayurvedic formulation known for its anti-inflammatory and antioxidant properties, including PLA^2 inhibition(47). Additionally, gene expression analysis showed that KB down regulates the Cacna1b gene, It encodes the α1 subunit of the $Cav^{2.2}$ calcium channel, which plays a role in pain signaling. Excess calcium influx via these channels promotes neuronal excitability and pain transmission. By reducing Cacna1b expression, KB may modulate neuronal excitability and diminish pain sensations. These findings suggest that KB offers dual benefits: anti-inflammatory effects and direct modulation of neuronal pain mechanisms, making it a promising candidate for managing neuropathic pain.

Neuroinflammation, driven by glial cell activation and the release of cytokines and inflammatory mediators, leads to neural damage. When nerve damage occurs, neurons emit chemicals that activate microglia, causing them to change morphologically, migrate to the site of the injury, and proliferate more—a process known as "microgliosis (48).

Microglial proliferation in the spinal cord was observed in mouse models of neuropathic pain after peripheral nerve injury, underlining their crucial involvement in pain genesis (49) Inhibiting microglial activation has been demonstrated to reduce allodynia and hyperalgesia, evaluation their importance in neuropathic pain pathways (50,51). Beyond neuropathic pain, microglial activation is implicated in stroke and neurodegenerative diseases and is influenced by voltage-gated calcium channels (VGCCs).

This study explores the effects of KB on BV-2 microglial cells, focusing on calcium (Ca^{2+}) channel activity. Notably, KB exhibited no adverse effects on cell viability, even in LPS-activated cells, demonstrating its safety and therapeutic potential. RT-PCR analysis revealed that 1 µg/mL LPS significantly upregulated Cacna1b expression in microglial cells, whereas no expression was observed under normal conditions. Pre-treatment with KB dose-dependently suppressed this upregulation, suggesting that KB inhibits calcium channels. Importantly, KB's ability to block calcium channels without compromising cell viability highlights its safety for prolonged use. These findings position KB as a promising formulation for preserving microglial health, preventing neurodegeneration, and managing neuropathic pain. Similar results were seen with the ayurvedic medication bacopa, a memory enhancer, In a cell-free test approach, this dramatically lowers the production of TNF-α and IL^6 from LPS-activated microglia and almost entirely blocks the activities of caspase 1 and 3, as well as MMP3(52).

The GC-MS analysis of KB identified 24 bioactive compounds, with high levels of n-decanoic acid, dodecanoic acid (lauric acid), tetradecanoic acid (myristic acid), pentadecanoic acid, and cis-vaccenic acid, which are likely responsible for its therapeutic properties. n-decanoic acid has been linked to improved seizure control in epilepsy models (53), while dodecanoic acid exhibits strong bactericidal activity, particularly against *Propionibacterium* acnes, making it useful for inflammatory acne treatment (54). Cis-vaccenic acid suppresses intestinal inflammation by increasing anandamide levels(55) and tetradecanoic acid has potential applications in targeted gene therapy for glioblastoma (56). Literature suggests that the presence of both short- and long-chain fatty acids contributes synergistically to its anti-inflammatory and neuroprotective effects (57), which may underlie KB ,reported neurological benefits.

Current study is limited to *invitro* screening, which, while valuable, does not fully replicate the complex physiological interactions present in living organisms. *In-vitro* models lack the ability to capture systemic effects, biodistribution, metabolism, and long-term therapeutic responses. Employing animal models for neuropathic pain would provide more comprehensive insights, as these models mimic the multifaceted nature of neuropathy, including behavioral, biochemical, and histological parameters. Such studies would help validate the therapeutic potential of KB under conditions that closely resemble human pathology.

Conclusion

The *in vitro* findings provide compelling evidence that KB holds significant promise as a therapeutic agent for the management of neuropathic pain. Ongoing *invitro* studies have helped establish pharmacological correlates for its traditionally documented clinical effects. KB's antioxidant properties, its inhibition of pro-inflammatory enzymes such as phospholipase A^2 (PLA_2), and the modulation of pain-related gene expression suggest a multifaceted mechanism of action capable of targeting key pathways involved in neuropathic pain.

Importantly, KB has been widely used in traditional medicine for centuries, and its safety is well established through both historical use and preliminary scientific evaluation. Its favorable safety profile observed in cellular models further supports its potential for clinical translation. Given the complexity and treatment resistance often associated with neuropathic pain, KB may offer an innovative and integrative approach to pain management. However, further research—particularly well-designed clinical trials—is essential to validate its efficacy in human populations.

Acknowlegement

The authors wish to thank SIMATS and Neoscience lab Chennai, for enabling the facilities to carry out the research

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