Bacopa as Crude Extracts Improve Outcomes in Spinal Cord Injuries in Rodent

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ABSTRACT

BACKGROUND
Spinal cord injury is a major cause of disability with most treatment modalities still in open question. Some alternative medicine treatments are moving into preclinical trials worldwide. Brahmi is used in the Ayurvedic system of medicine for centuries. Traditionally used as a brain tonic to enhance memory, learning and concentration, and in some previous work shows neural regeneration in hippocampal cells. This study was done to look at the role of one of these neuroprotective plant products in spinal cord injury based on the hypothesis that if neural stem cells differentiate with more dendrites in vitro, it may do the same in vivo with clinical benefit.

METHODS
Controlled spinal cord injury in form of hemisection was done in rodents that were housed in the animal house of Sikkim Manipal Institute of Medical Sciences, followed by administration dose of bacopa. The comparative study was done by looking at the improvement in hind limb movement by known study methods which were modified for our study.

RESULTS
The result of rodents shows a more rapid recovery through catwalk and IBB scoring treated with crude bacopa than control one. The histological evaluation was also looked after sacrificing the rodent at the end of the study which corroborates the functional findings of improvement in neural regeneration in the spinal cord.

CONCLUSIONS
Bacopa crude extracts seem to be beneficial in spinal cord injury. Evaluation of different bacopasides to find which component of the crude product is beneficial.

KEY WORDS
Bacopa, Spinal Cord Injuries, Animal Experimentation, Gait Analysis

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BACKGROUND

Spinal cord injury leads to significant disability. Experimental animal studies need different tools to assess Catwalk gait analysis for damaged spinal cord and functional recovery of the same. Spinal cord injury leads to impairment of motor neurons in the spinal cord and nerve roots. Bacopa has been used in Indian Medicine (Ayurvedic) for improving cognition. The typical usage is as a food item or as crude extract as used in alternative medicine. Various basic science research and clinical work have provided discrepant information. Probably the authors use a null hypothesis for the experiment decided to look at the morphological changes of neural cells when Bacopa is added in non-toxic doses to the neuronal stem cell cultures. We observed in our previous study that Bacopa crude extracts when added to neural stem cell cultures cause differentiation of the cells with increased dendrite formation than the controls.1

BM extracts help to repair damaged neurons and exhibit a neuroprotective effect against oxidative stress in the hippocampus of the rat brain.2 Using this as a start point a hypothesis was generated that dendrite formation may be useful for the repair of the central nervous system. A small animal model that gives functional information on neural tract damage and regeneration in spinal cord injury was derived and monitoring of the functionality of the hind limb was done and studied. The phases of spinal cord injury have been divided into 3 phases Acute, Subacute and Chronic by Pajer.3 The PubMed search with the following MeSH items "(Bacopa[Mesh]) AND "Spinal Cord Regeneration/drug effects[Mesh]" yielded no results till 22nd December 2021. Then believe the information obtained from this experiment is novel and useful for future treatment of the neural injury.

METHODS

Design of experiment (animal model design and statistical design). The previous tissue culture experiment done by us helped to titrate an appropriate dose that is functional yet not toxic for rodents, both internal control i.e. hemisection of spinal cord created and functional tract and limb and one non-functional tract and limb was created.1 A preclinical animal trial was conducted on the injured spinal cord of rodents and evaluated the walking gait. Henceforth we use the term catwalk since a correlation was made for the rodent to walk in a defined route. Analysis for neural regeneration and functionality. It was further worked up by the histological appearance of the injured area after a specific period.

Statistical Design

A factorial design for treatment response was used. It was based on the guidelines given in “Guidelines for the Design and Statistical Analysis of Experiments in Papers Submitted to ATLA” M Festing ATLA. Sample size and error rate calculated using R statistics,6 details are given below in Table 1.

Simon 2-stage Phase II design

Unacceptable response rate: 0.1 Desirable response rates: 0.6

<p>| Error rates: alpha = 0.05; beta = 0.2 |
|---|---|---|---|---|---|---|</p>
<table>
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<tr>
<th>r1</th>
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<th>EN</th>
<th>p0</th>
<th>PET (p0)</th>
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<td>2</td>
<td>8</td>
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<td>0.010</td>
</tr>
<tr>
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<td>2</td>
<td>2</td>
<td>6</td>
<td>3.813</td>
<td>0.729</td>
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The process flow chart is given below in Fig. 1

Preparation of Crude Bacopa Extract

Crude bacopa is available commercially extracted in dry powder form by the brahmi capsule manufactured by the Himalaya Drug Company. A stock solution of BM was made by mixing one capsule (250 mg) in 1000 ml of distilled water and sonicated for 2 min at 1 cycle and 80 % amplitude [ultrasonic processor UP50H (50 watts, 30 kHz)]. Then the solution was filtered with a microsyringe filter and sterilized with a microspore filter size of 45 microns. Then required dilution of 80 mg/kg body weight was orally administered to the rodent.

Design of Rodent Spinal Cord Injury

There are various methods to create standardized spinal cord injury so that all mice have the same type of injury. The initial animal model for spinal cord injury was created by Allen AR5 in 1911. It was a crush injury model based on controlled contusion by weight drop and dislocation of the spinal cord.6 They provided a comprehensive review of different spinal cord injury models. These include Contusion models (New York University (NYU)/Multicenter Animal Spinal Cord Injury Study (MASCIS) impactor, Ohio State University (OSU) impactor, Air gun impactor and Infinite horizon (IH) impactor); Compression models (Clip compression, Calibrated forceps compression, Balloon compression, Spinal cord strapping); Distraction models (Harrington distractor, University of British Columbia (UBC) multi mechanism device and the University of Texas at Arlington (UTA) distractor); Dislocation model; Transection model(Full transection, Partial transection); Chemical models. We chose the transection model5 because we got an internal control to compare the 2 hind limb movements. The neurosurgeon in the team designed the following experimental procedure to have some type of trauma in all mice. The surgical manoeuvre was practised repeatedly till the surgical operation was sure that all rodents had the same type of injury. The mice were anaesthetized by intraperitoneal injection of ketamine and diazepam and were given air/oxygen at a low flow rate by an improvised rodent mask. The dorsal skin was incised on a warm rodent surgical suite (manufactured by Kent Inc.). The lamina was cut using bone scissors (manufactured by Kent Inc.). The controlled injury was done from the dorsal aspect by hemisection (crush) of a column by inserting a pointed forceps with one limb outside the cord and one limb of the forceps in the middle of the spinal cord. The entire process was done with an operating cum dissecting microscope (model no STEMI 305 manufactured by Carl Zeiss). IAEC approved the protocol for the same.

Method of Bacopa Administration

Bacopa was prepared and made at 80 mg/kg body weight. Using rat gavage tube was administered orally once a day and...
then rodents were given normal feed. Rodents were kept in the animal house of Silkkim Manipal Institute of Medical Sciences with 12 hrs light and dark cycle. The Institutional Ethical Committee approved the same protocol.

Follow-up and recording of the rodent in a standardized format.

For gait analysis, the feet of the rodent was painted. The footprint of the rodent was taken up on a white sheet placed in a contraction made of wood with a clear glass cover on it to see through the whole track. The track was 110 cm long and 10 cm wide. Here the rodents were trained to cross the walking track before the spinal injury. All the 12 test animals were made to walk over the walking track one day before spinal cord injury and after 12 hrs. Spinal injury for the next 6 days continued and then gait was recorded finally once after 14 days. The pattern of gait analysis is based on Medinaceli. An additional t-test was performed by the data which was procured from catwalk imprints.

Data Analysis of three to four prints of each foot of right and left side of fore and hind limb were used for measurement. The variables are as follows:

**Distance of Opposite Foot (TOF).** This is the orthogonal projection of distance from the tip of the experimental foot to the tip of the following contralateral toes that was called experimental TOF. Normal "to opposite foot" (NTOF) was defined inversely. The longest TOF for each side was used for the experiment.

**Print Length (PL).** The length of the footprint was measured on the normal and experimental sides.

**Total spreading of toes (TS).** The linear distance from the centre of the print of the first toe to the print of the fifth toe was measured on both sides.

**Distance between intermediate toes (IT).** The distance between the second and fourth toes was measured to the nearest on each side.

The quantification given by Medinaceli is given in the equation.[9] The normal functional index of the injured cord of these four variables were measured on each side using:

\[
\text{SFI} = \frac{\text{ETOFT} - \text{NTOF}}{\text{NTOF}} + \frac{\text{NPL} - \text{EPL}}{\text{EPL}} + \frac{\text{ETS} - \text{NTS}}{\text{NTS}} + \frac{\text{EIT} - \text{NIT}}{\text{NIT}} \times \frac{220}{7}
\]

Note that this is Sciatic Functional Index. The final motor nerve transmits signals for movement after the spinal cord.

**Histomorphological Study**

The basis of our histological study was that the epicentre of injury had telltale signs.[9] The lesion was assessed based on the visual image at 10x, 20x and 40x. Briefly, sagittal spinal cord sections from 5 mm rostral to 5 mm caudal the lesion sites were taken. A random start section for each sample was chosen and was stained with haematoxylin and eosin (H&E) and photographed at 10x, 20x and 40x (Leica ICC50 HD system; Leica). The lesion area, including both the cavity and surrounding damaged tissue, was then outlined in the LAS EZ Image program (Leica) and the resultant findings tabulated. The average lesion area was assessed for each microscopic field of injury.

**RESULTS**

Exceptions: in some cases of substantial deficit, the animals walked on the front foot and no measures could be made on the experimental side. Following value from Medinaceli,[9] arbitrary values used were: ETOF = one-third of the distance between two normal footprints (normal side stride), NTOF = two-thirds of that distance, EPL = 32mm, ETS=10mm, and EIT = 10mm. Similarly, when in spite of placing of the hind limb, ETS and EIT were not visible on the paper because of the paralysis and could not be measured; an arbitrary value of 10mm was attributed to each.

The catwalk gait analysis was performed on the rodents before and after the surgery, and the bacopa orally administered to rodents showed a significant recovery rate. The dragging of the hind part of the body (based on the front limb) continued for not more than the 4th day of observation after surgery and administration of bacopa. Weight-bearing capacity was seen to be steadily increasing in the orally administered bacopa rodents than in the Sham Operated Rodents, where the SFI did not significantly shift towards the normal value. The animals were significantly impaired on the first post-operative day and showed the same pattern till the 14th day of data collection. The P-value between SHAM and Bacopa was < 0.01 which portrayed significance and was further established by the histomorphological study of the spinal cord of the test rodents.

Recovery index data for the spinal injury from four variables from crush injury is shown in table 2.

**Histomorphological Study**

**Morphological Changes**

All animals that were alive within one week of surgery were followed up for 6 months and then sacrificed to see microscopic/histologic changes. Thus the 2 controls (sham surgery) could not be included. This experimental workup looked at the long term morphology of neural healing after the functional recovery of hind limb movement in the rodents. The features that were considered important in cell injury and repair were listed. The three major ones were first, gliosis or any other form of replacement by neurocytes. Secondly, the infiltration by inflammatory cells was classified. Thirdly, regeneration of neural cells was looked at. A non-quantitative subjective comparison was possible in a trinary format of significant presence, mild presence and absence.

**Gliosis**

All controls with sham surgery had significant gliosis (filling greater than 50 % of high power field). The group that was bacopa administered had the presence of gliosis but it was mild (Less than 10 % of high power field).
**Inflammatory Cell Infiltrate**

The inflammatory cells were significantly present in all the members of the control group. Inflammatory cell infiltrate was absent in 3 bacopa administered groups and insignificant presence in 2 of the bacopa administered group. Neural regeneration: Neural heads were present in 2 of the bacopa administered group but were not significant. In the bacopa administered group, there was a significant increase in the number of neural heads and more dendrite formation. The representative figures are given below.

**DISCUSSION**

Hemisectioning and Animal Observation: The rodents received hemisection at the level of the T9 and T10 vertebra. The sectioning led to impairment of the physical functioning of the hind limbs. More so the cellular development of the limb was also lagging, post sectioning. The sectioning resulted in equal severity in damage to both hind limbs, irrespective of the side of the site of injury.

SFI and Histomorphological: The SFI indexing portrayed that SHAM operated rodents did not show recovery as compared to the group that received oral administration of bacopa. Moreover, the latter group showed catalytically faster recovery rates along with the neural regeneration (elucidated by the histomorphological slides observed).

The bacopa oral administration which is already in use for the recovery from cognitive impairment can also be tried on traumatic neural injury as a case-control study. The changes in structure because of BM extracts in the neurons have also been earlier demonstrated with electron microscopic evaluation.[10] Bacopa has also been found to have wound healing activity[11] and also shows a broad spectrum of antibacterial activity.[12] The use of BM extracts significantly decreases lipid peroxidation, and lipofuscin deposition, and reduces the structural derangements in the hippocampus.[13] The higher doses of BM (20, 40, and 80 mg/kg) over a long duration of 4 to 6 weeks have shown dendritic formation in Wister rats’ basolateral amygdala neurons.[13]

Catwalk analysis of gait in repair in hind limb injury shows reliable results for showing velocity and static gait alteration.[14] In behaviour test tool, walking pattern analysis from which nerve functional index has been calculated.[8,15] The changes after neurotmesis demonstrated using catwalk are smaller base-of support (BOS), less contacted area on the ground and coordination. The results of BOS after-show decreased in other types of injury but increased in spinal cord injury.[16]

**CONCLUSIONS**

In spinal cord injury, bacopa may have a beneficial role. Further clinical trials in humans may be undertaken to know its usefulness on them.

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**Disclosures**

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