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Original article

Preventive potentials of piperlongumine and a *Piper longum* extract against stress responses and pain

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ABSTRACT

Aim: To compare stress resistance increasing and analgesic activities of piperlongumine and a methanolic *Piper longum* fruit extract (PLE).
Methods: Efficacies of a single and repeated daily oral doses (1–256 mg/kg/day) of PLE, piperlongumine, and 50 mg/kg/day doxycycline against foot shock stress triggered alteration in body weights and core temperatures, and of their 11 daily doses on antidepressants like activity in tail suspension test and on pentobarbital induced sedation in male mice were compared. In another experiment, analgesic activities of single and repeated daily 5 mg/kg oral doses of piperlongumine and PLE in mice hot plate test and in acetic acid induced writing tests were compared with those of aspirin and doxycycline.
Results: After their single oral doses no effects of piperlongumine or PLE or doxycycline were observed in the footshock stress induced hyperthermia test or in hot plate test. However, significant effects of piperlongumine and PLE in both the tests were observed after their 5 or more daily doses. Both of them also dose dependently suppressed daily handling and repetitive testing triggered alterations in body weights and core temperatures. Their doxycycline like antidepressant activity in tail suspension test and aspirin like analgesic effects in acetic acid writhing test were observed after their 11 daily 5 mg/kg oral dose.
Conclusion: Piperlongumine is another bioactive secondary metabolite of *P. longum* and other plants of piper species with stress response suppressing, analgesic, and anti-inflammatory activities. Its bactericidal activities can also contribute to its therapeutically interesting bio-activity profile.

1. Introduction

Piper longum Linn, commonly known as long piper or Indian long piper, is a plant of Piperaceae family cultivated for harvesting its fruits used in several Asiatic and other countries as a spices and seasoning. Its diverse medicinal uses have also been known in Ayurvedic and other traditionally known systems of medicine and health care since antiquity.[1] Like several other plants widely used in modern Indian system of medicine, *P. longum* is now often pharmacologically classified as an adaptogenic or stress response regulating medicinal herbs with a broad spectrum of therapeutically interesting bioactivities.[2–5] Piperlongumine, piperine and other amide alkaloids with pungent tastes have been identified as the major and structurally unique bioactive secondary metabolites of the plant.[6,7] However, these and numerous other alkaloidal amides of *P. longum* are also encountered in varying quantities in other plants of the Piperaceae family which are also often used in Chinese and other traditionally known systems of medicine.[8,9] Piperlongumine is now attracting considerable attention of modern drug discoverers as a lead structure for obtaining functionally novel drug leads against inflammatory disorders and cancer.[10,11] However, most extracts of *P. longum* and other plants of the Piperaceae family often used in modernized versions of Ayurvedic

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formulation are in general analytically standardized, or analytically characterized, by their piperine contents only.[12,13]

Trikatu is one such herbal mixture containing equal parts of *P. longum* and *P. nigrum* fruits and *Zingiber officinalis* root powder.[14] This mixture is often used in many Ayurvedic formulations commonly prescribed for treatments of gastric and abdominal disorders, asthma, bronchitis, coughs, dysentery, pyrexia, insomnia, colic and intestinal infection. Diverse stress response regulating potentials of all the three plant used in this mixture have been reported,[15] and such properties of *P. longum* extracts and their analgesic, ulcer protecting and diverse other therapeutically interesting bioactivities of piperine in animal models are also well known.[16–19] However, as yet no reports on the role of piperlongumine in stress response modulating, pain relieving, and diverse other traditionally known medicinal uses of extracts *P. longum* and other plants of Piperaceae family have appeared.

Piperlongumine and piperine are structurally analogous bioactive molecules and both of them have been reported to possess antimicrobial activities.[20] It is now becoming increasingly apparent that gut microbiota play a crucial role in regulating physiological stress responses,[21] and that depending on their doses and treatment regimen used, bactericidal and other agents with modulating effects on gut microbial ecology can have diverse health benefits.[22,23] For example, appropriate doses and treatment regimen of doxycycline like antibiotics can suppress physiological stress response and can reset the neuro-hormonal status regulated by gut microbiota.[24] Gastric ulcer protective, anticonvulsant, antidepressant, neuro-protective and other brain function modulating activities of the antibiotic have been reported also.[25–28] Therefore, it was of interest to experimentally very the possibility that like medicinally used *P. longum* extracts and doxycycline, pure piperlongumine also possess stress response suppressing and analgesic activities. Results of the very first experiments conducted to experimentally verify such possibilities are described and discussed in this communication.

The reported experiments constitute a part of our ongoing efforts directed towards translating Ayurvedic therapeutic principles in terms of molecular concepts of modern medicine and to obtain therapeutic leads from traditionally known medicinal plants potentially useful for prevention and cure of psychosomatic disorders commonly associated with systemic inflammation.[29] In this study, a pharmacologically well characterized mouse bioassay procedure evolving from our efforts to estimate therapeutically interesting doses and dosing regimen of stress response regulating medicinal plants and their bioactive constituents[30–32] was used for comparing the efficacies of doxycycline like adaptogenic efficacies of piperlongumine and a commercially available *P. longum* extract in mice. In a further experiment, aspirin like analgesic efficacies of their stress response suppressing doses and treatment regimen were compared in a slightly modified version of the bioassay procedures used in the pilot dose finding experiments.

2. Materials and methods

2.1. Animals

Adult male Swiss albino mice (25 ± 5 g) were acquired from Central Animal House of Institute of Medical Sciences, Banaras Hindu University (Registration Number: 542/AB/CPCSEA), and they were acclimatized to laboratory conditions for one week before starting the experiments. For experimental purposes, six animals per group were housed in polypropylene cages (28 × 19 × 12.5 cm) with saw dust beddings and free access to standard rodent diet and water. They were maintained at 25 ± 1 °C ambient temperature and relative humidity of 50 ± 10% and 12:12 h light and dark cycle (light on at 06:00 and off at 18:00). Principles of laboratory animal care (NIH publication 85-23, revised in 1985) guidelines were always followed and before start of the experiments, approval from Central Animal Ethical Committee of the University was obtained (Dean/2014/CAEC/729, dated 07 August 2014).

2.2. Plant extract, drugs and chemicals

Together with their analytical characteristics, the *P. longum* fruit extract analytically characterized to contain 1.75% piperine (PLE) and almost pure piperlongumine (99.33%) isolated from *P. longum* roots used in this study were generously supplied by Sami Labs Limited, Bengaluru, India. The extract PLE is a methanolic extract of dried and powdered fruits of *P. longum* fruits and purity of piperlongumine and piperine contents of the PLE sample were established by HPLC using acetonitrile and water as mobile phase.

Doxycycline was acquired from Sigma Aldrich, Bengaluru, India; Pentobarbitone sodium from Loba Chemicals Pvt. Ltd., Mumbai, India; Aspirin from HiMedia Laboratories Pvt. Ltd., Mumbai, India; and Carboxymethyl cellulose (CMC) from Central Drug House, Delhi, India. All other chemicals and reagents used in this study were of highest purity commercially available in India.

2.3. Animal grouping and drug administration

In each of the two pilot experiments conducted to estimate stress response modulating doses and dosing regimen of PLE and piperlongumine, seven groups of six animals each were used. One of them (vehicle treated control group) was treated daily with 0. 3% CMC (10 ml/kg/day), and another one with 50 mg/kg/day doxycycline suspended in CMC for 11 consecutive days. Other five groups were similarly treated either with graded oral daily doses (1, 4, 16, 64 and 256 mg/kg) of piperlongumine or PLE suspended in CMC. In these experiments, 1 h after oral treatments on days 1, 5, 7 and 10 of the experiments all animals were subjected to foot shock stress triggered hyperthermia tests, and 1 h after the treatments on the 11th day of the experiments they were subjected to tail suspension test for potential antidepressant. One day thereafter all groups (without further treatments) were subjected to pentobarbitone sleep tests for assessing longer lasting effects of treatments on brain functions or on drug metabolizing enzymes. Further details of these experiments are summarized in Fig. 1a.

In a further experiment conducted to compare aspirin or doxycycline like analgesic effects of 11 daily oral doses of 5 mg/kg PLE, or of piperlongumine, six groups of six animals each were used. Hereupon, one control group (Control-HPT) was treated with CMC but not subjected to hot plate test on days 1, 5, 7 and 10 of the experiment and another one (Control + HPT) was treated with CMC and also subjected to hot plate test on those days. The remaining four groups in this experiment were daily treated either with 20 mg/kg/day aspirin, or with 50 mg/kg/day doxycycline or with 5 mg/kg/day piperlongumine or PLE and subjected to hot plate test on those days. The animals used in this experiment were pre selected ones for their sensitivity to hot plate test. Pre-selection of the animals were done one day before the start of the experiment. For such purposes, animals were placed on a hot plate maintained at 55 ± 1 °C and their reaction time was recorded. Only mice which reacted within 15 s and which did not show large variations in their response times when tested on four separate occasions (each 15 min apart) were used in the experiment. On the 11th day of this experiment, and 1 h after the days treatments, all animals of all groups were subjected to acetic acid induced writhing test for analgesics, and on the 12th day of the experiment to tail suspension test for antidepressants. Further details of this experiment are summarized in Fig. 1b.

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