Research Article

Histopathological Evaluation of The Neuroprotective Effect of Andrographolide–Piperine Combination in Streptozotocin-Induced Alzheimer's Disease

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Abstract

Streptozotocin (STZ) is a glucosamine-nitrosourea derivative that can interfere with insulin-mediated signalling in the brain and disturb neuronal glucose utilisation. These alterations are associated with progressive cognitive dysfunction and several pathological features resembling Alzheimer's disease (AD). When administered intracerebroventricularly (ICV), STZ produces central metabolic and neuronal disturbances, including increased amyloid-β (Aβ) deposition and impairment of learning and memory. Andrographolide (ANDRO), a naturally occurring diterpenoid obtained from Andrographis paniculata, has attracted attention because of its multiple pharmacological effects, particularly its antioxidant, anti-inflammatory, anti-cholinesterase, and neuroprotective activities. Therefore, the present investigation was designed to determine whether ANDRO could protect against the behavioural and neurochemical abnormalities produced by ICV-STZ in Wistar rats. For induction of the experimental AD-like condition, the animals were administered STZ (3 mg/kg) twice through the ICV route. Following model induction, ANDRO was administered orally at doses of 15, 30, and 60 mg/kg, whereas donepezil (5 mg/kg, orally) was used as the standard treatment for 14 consecutive days. Behavioural performance was monitored at predetermined weekly intervals to assess the progression of cognitive and functional deficits. At the end of the experimental period, i.e., on day 21, the animals were sacrificed and hippocampal tissue was collected for biochemical and neurochemical analyses. The investigated parameters included malondialdehyde (MDA), nitrite, reduced glutathione (GSH), superoxide dismutase (SOD), catalase, and acetylcholinesterase (AChE). The hippocampal concentrations of the inflammatory mediators interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α), together with the neurotransmitters glutamate and γ-aminobutyric acid (GABA), were also determined. In addition, amyloid-β1-42 (Aβ1-42) and phosphorylated tau (p-tau) were quantified to assess AD-associated pathological alterations. The experimental findings demonstrated that exposure to ICV-STZ resulted in pronounced behavioural impairment accompanied by disruption of hippocampal oxidative balance, cholinergic function, inflammatory status, and neurotransmitter homeostasis. Administration of ANDRO ameliorated these STZ-associated alterations, indicating a protective influence on both behavioural and biochemical outcomes. ANDRO treatment also produced a reduction in the hippocampal burden of Aβ1-42 and p-tau, suggesting that its protective activity may extend beyond symptomatic improvement to modulation of important pathological features associated with AD-like neurodegeneration. Overall, the histopathological findings indicate that ANDRO exerts a protective effect against ICV-STZ-induced hippocampal neuronal degeneration and loss. The improved preservation of hippocampal neuronal architecture, particularly following higher-dose ANDRO treatment and ANDRO–piperine combination therapy, supports its potential neuroprotective role in experimental AD-like neurodegeneration. These findings warrant further investigation of ANDRO, alone and in combination with piperine, as a potential phytotherapeutic approach for attenuating structural neuronal damage associated with AD-like pathology.

Keywords: Andrographolide, Piperine, Alzheimer's disease, ICV-STZ, Neuroinflammation, Oxidative stress, Cognitive dysfunction, Histology.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia worldwide, accounting for a substantial proportion of dementia cases and representing an increasingly significant socioeconomic and public health burden as the global population continues to age [1]. AD is a progressive and multifactorial neurodegenerative disorder characterised by a complex interaction of pathological, biochemical, and molecular abnormalities. The major neuropathological hallmarks include extracellular deposition of amyloid-β (Aβ) plaques, intracellular accumulation of neurofibrillary tangles composed predominantly of hyperphosphorylated tau protein, impairment of cholinergic neurotransmission, synaptic dysfunction, neuroinflammation, oxidative stress, mitochondrial abnormalities, and progressive neuronal loss [2]. Although these pathological changes occur throughout the brain, the hippocampus and cerebral cortex are particularly vulnerable, resulting in substantial impairment of learning, memory, spatial orientation, and other cognitive functions. The progressive nature of these pathological changes ultimately leads to deterioration in activities of daily living and substantially reduces quality of life. Despite extensive research into the molecular mechanisms underlying AD, currently approved pharmacological treatments, including acetylcholinesterase inhibitors and N-methyl-D-aspartate (NMDA) receptor antagonists, primarily provide symptomatic improvement and have limited effects on the underlying neurodegenerative process [3]. Therefore, there remains a considerable need for therapeutic approaches capable of simultaneously targeting the interconnected oxidative, inflammatory, cholinergic, metabolic, and neurochemical abnormalities involved in AD progression.

Increasing evidence suggests that disturbances in brain insulin signalling may represent an important contributor to the pathophysiology of sporadic AD. Impaired insulin signalling within the central nervous system can adversely affect glucose utilisation, synaptic plasticity, neuronal survival, mitochondrial function, and memory formation [4–6]. This concept has contributed to the description of AD as a form of "type 3 diabetes," reflecting the presence of brain insulin resistance and impaired insulin-mediated neuronal signalling. Intracerebroventricular administration of streptozotocin (ICV-STZ) in experimental animals induces several of these central abnormalities and produces a characteristic AD-like phenotype. ICV-STZ administration has been reported to cause impaired brain insulin signalling, oxidative stress, mitochondrial dysfunction, cholinergic disturbances, altered neurotransmitter homeostasis, and neuroinflammatory responses, ultimately resulting in synaptic dysfunction, neuronal degeneration, and progressive cognitive impairment [7,8]. Importantly, the ICV route produces predominantly central nervous system effects and avoids the marked peripheral metabolic disturbances associated with systemic STZ administration. Consequently, ICV-STZ-induced neurodegeneration has become a widely employed experimental model for investigating the mechanisms associated with sporadic AD and for evaluating potential neuroprotective, antioxidant, anti-inflammatory, and cognition-enhancing agents [9,10].

The multifactorial nature of AD provides a rationale for investigating pharmacological interventions that can act simultaneously on several pathological pathways. In this context, andrographolide has attracted considerable interest as a naturally occurring neuroprotective compound. Andrographolide is a major labdane diterpenoid isolated from Andrographis paniculata and possesses a broad range of pharmacological activities, including antioxidant, anti-inflammatory, anti-apoptotic, and neuroprotective effects [11,12]. Experimental studies have demonstrated that andrographolide can attenuate oxidative damage, reduce the generation of reactive oxygen species, modulate pro-inflammatory signalling pathways, and protect neuronal cells against various forms of cellular injury. In particular, inhibition of nuclear factor-kappa B (NF-κB)-mediated inflammatory signalling may contribute to its ability to reduce the production of pro-inflammatory mediators and limit neuroinflammatory damage [10,11]. In addition, its reported ability to attenuate neuronal apoptosis and oxidative stress provides a mechanistic basis for investigating its protective effects in neurodegenerative disorders. These pharmacological properties are particularly relevant to AD, in which oxidative stress, chronic neuroinflammation, mitochondrial dysfunction, and neuronal apoptosis interact to promote progressive synaptic and neuronal damage. However, despite its promising biological activity, the therapeutic application of andrographolide may be restricted by its relatively poor oral bioavailability, which has been associated with extensive metabolism and limited systemic exposure [13].

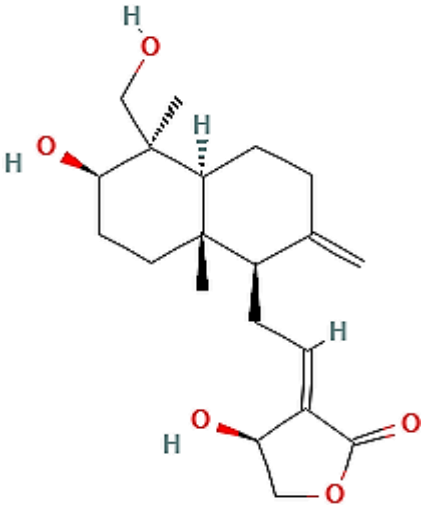
Piperine, the principal pungent alkaloid present in Piper nigrum (black pepper), has received considerable attention as a natural bioenhancer capable of increasing the bioavailability of several drugs and phytoconstituents [14,15]. Piperine can influence pharmacokinetic processes by inhibiting intestinal and hepatic drug-metabolising enzymes and modulating drug transport mechanisms, including cytochrome P450-mediated metabolism and P-glycoprotein-associated efflux [14,15]. Such effects may increase the systemic exposure and pharmacological availability of concurrently administered compounds. In addition to its bioenhancing properties, piperine itself possesses antioxidant, anti-inflammatory, and neuroprotective activities, suggesting that its effects may extend beyond pharmacokinetic enhancement [16,17]. Piperine has therefore been proposed as a potentially useful adjunct for improving the therapeutic effectiveness of bioactive phytoconstituents. Co-administration of piperine with andrographolide may consequently provide a dual advantage by improving the availability of andrographolide while simultaneously contributing to antioxidant and neuroprotective activity. This pharmacological rationale raises the possibility that the combination may provide greater protection against the multiple pathological processes associated with AD-like neurodegeneration than andrographolide administered alone [18].

The potential therapeutic relevance of this combination is further supported by the interconnected nature of oxidative stress, neuroinflammation, cholinergic dysfunction, neurotransmitter imbalance, and neuronal degeneration in AD. Excessive oxidative stress can promote lipid peroxidation, depletion of endogenous antioxidant defences, mitochondrial dysfunction, and neuronal injury, while persistent neuroinflammation can amplify oxidative damage and promote the release of pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α). Concurrently, impaired cholinergic neurotransmission, reflected by alterations in acetylcholinesterase activity, contributes to deficits in learning and memory. Alterations in excitatory and inhibitory neurotransmitters, including glutamate and γ-aminobutyric acid (GABA), may further contribute to neuronal dysfunction and cognitive impairment. Therefore, an intervention capable of modulating several of these pathways simultaneously may have potential for attenuating the complex pathological consequences of ICV-STZ administration.

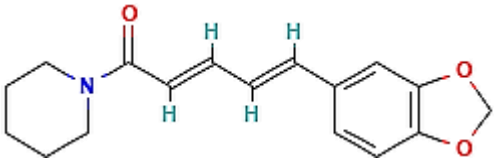
Despite the established individual pharmacological properties of andrographolide and piperine, limited experimental evidence is available regarding their combined neuroprotective effects in an in vivo model of AD-like pathology [10,19,20]. In particular, the effects of this combination on behavioural dysfunction, oxidative stress, cholinergic activity, neuroinflammatory mediators, neurotransmitter alterations, and hippocampal structural integrity following ICV-STZ administration require further investigation. The hippocampus is particularly relevant because of its central role

in learning and memory and its vulnerability to oxidative and inflammatory neuronal injury. Histopathological assessment of hippocampal tissue can therefore provide additional structural evidence to complement behavioural and biochemical findings and may help determine whether pharmacological treatment is associated with preservation or restoration of neuronal architecture.

Accordingly, the present study was designed to systematically evaluate the effects of andrographolide, administered alone at different doses and in combination with piperine, on ICV-STZ-induced alterations in adult Wistar rats. The study specifically investigated changes in locomotor and exploratory activity, spatial learning and memory, anxiety-related behaviour, oxidative stress and antioxidant defence systems, cholinergic function, neuroinflammatory mediators, and the levels of the neurotransmitters GABA and glutamate. In addition, hippocampal histopathological changes were examined to determine the extent of neuronal degeneration and the degree of structural preservation following treatment. The neuroprotective effects of andrographolide alone and in combination with piperine were further compared with those produced by the standard anti-Alzheimer's drug donepezil. Collectively, these investigations were undertaken to determine whether piperine-mediated bio enhancement could potentiate the neuroprotective effects of andrographolide and provide broader protection against the behavioural, biochemical, neurochemical, inflammatory, and histopathological consequences of ICV-STZ-induced AD-like neurodegeneration.



Andrographolide



Piperine

2. Materials and methods

2.1. Experimental animals

Adult male Wistar rats weighing 250–280 g were obtained from the Central Animal House of the ISF College of Pharmacy, Moga (Punjab). The animals were housed in polyacrylic cages under standard laboratory conditions, with a temperature maintained at 22 ± 2 °C, relative humidity of 55–60%, and a 12-hour light/dark cycle. The rats were provided a commercial pellet diet and had free access to drinking water throughout the experimental period. To maintain consistency and minimize the influence of circadian variations, all behavioural tests were conducted during fixed time periods, i.e., between 9:00–11:00 a.m. and 2:00–5:00 p.m. The experimental protocols were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) under protocol number ISFCP/IAEC/CPCSEA/Meeting No. 29 /2025/ Protocol No.431 at 11:00 a.m. Following ethical approval, the animals were maintained in the institutional animal house for the subsequent six months. All experimental procedures were conducted in accordance with the guidelines prescribed by the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India [21].

Drugs and chemicals

The drugs, kits, and reagents used in the present study included andrographolide, piperine, streptozotocin, ELISA kits, and other analytical-grade reagents. Andrographolide, used as the test compound, was procured from Sigma-Aldrich

(St. Louis, MO, USA) with a purity of 98% and a CAS number of 5508-58-7. Piperine was also obtained from Sigma-Aldrich with a purity of 97% and a CAS number of 94-62-2. Streptozotocin was procured from Sigma-Aldrich. ELISA kits for the estimation of interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α) were obtained from Krishgen Biosystems, India, and Bio. Sys (Whittier, CA, USA). All other reagents used in the study were of high-purity analytical grade. To ensure stability, reproducibility, and efficacy, all drug solutions and reagent preparations were freshly prepared immediately before use

2.2. Surgical procedure for intracerebroventricular administration of streptozotocin

Experimental Procedures

For the induction of an Alzheimer's disease-like phenotype, intracerebroventricular (ICV) administration of streptozotocin (STZ) was performed according to previously reported methods [19,22]. Adult male Wistar rats were initially acclimatized to the laboratory environment and maintained under standard experimental conditions before commencement of the study. The animals were appropriately anaesthetised with ketamine hydrochloride (70 mg/kg, intraperitoneally) and carefully positioned on a stereotactic frame to ensure accurate and reproducible administration of STZ into the lateral ventricles. After securing the animal, the scalp was prepared and a midline sagittal incision was made to expose the skull and identify the Bregma as the anatomical reference point. Two bilateral burr holes were subsequently drilled at predetermined stereotactic coordinates corresponding to the lateral ventricles, namely 0.8 mm posterior to Bregma, 1.5 mm lateral to the sagittal suture, and 3.6 mm in depth from the skull surface. A freshly prepared solution of streptozotocin at a dose of 3 mg/kg body weight, dissolved in sterile normal saline, was administered intracerebroventricularly through the respective openings, with a total injection volume of 20 µL [10,19]. Following administration, the injection site was appropriately managed and the animals were carefully monitored during the immediate postoperative period. To facilitate recovery and maintain adequate nutritional support after surgery, the animals were administered 1.5 mL of sweetened milk orally through an intragastric tube once daily for four consecutive days or until normal feeding behaviour was restored. The animals were subsequently observed for general health, behavioural changes, food and water intake, and recovery from the surgical procedure before commencement of the treatment regimen. Following successful establishment of the ICV-STZ-induced experimental model, the animals were allocated to their respective experimental groups and treated according to the predefined study protocol. Behavioural, biochemical, neurochemical, inflammatory, and histopathological parameters were subsequently evaluated to determine the neuroprotective effects of andrographolide administered alone and in combination with piperine.

The animals were randomly assigned to eight groups (Total animals 60)

A. **Normal control-6**
B. **Piperine per se-6**
C. **ICV-STZ (3 mg/kg)-8**
D. **STZ + ANDRO (15 mg/kg, orally)-8**
E. **STZ + ANDRO (30 mg/kg, orally)-8**
F. **STZ + ANDRO (60 mg/kg, orally)-8**
G. **STZ + ANDRO (15 mg/kg, orally) + piperine (2.5 mg/kg, orally)-8**
H. **STZ + donepezil (5 mg/kg, orally; standard group)-8**

Histological assessment

2.3. Histological analysis

Histological examination of the brain tissue was performed according to the previously described method [24]. Briefly, the rat brains were collected and fixed in 5% formaldehyde to preserve the tissue architecture. The fixed tissues were subsequently processed and embedded in liquid paraffin wax. Serial sections of approximately 5 µm thickness were prepared using a microtome and stained with hematoxylin and eosin (H&E). The stained sections were examined microscopically for histopathological alterations using a fluorescence microscope (Model 102 M, Motic Microscopes, China) at 40× magnification.

3. Results

3.1. Effect of andrographolide and piperine treatment on histopathological deficits in streptozotocin-treated rats

The effect of andrographolide and piperine treatment on histopathological alterations associated with i.c.v.-streptozotocin (STZ)-induced Alzheimer's disease (AD) in adult rats was evaluated in the hippocampal region. Histological sections were examined under a fluorescent Motic microscope (Model BA-310) at 40× magnification, and the representative photomicrographs are presented in Figure 1. The normal control group exhibited well-preserved hippocampal architecture, with neurons displaying relatively normal morphology and an orderly arrangement. In

contrast, the i.c.v.-STZ-treated group showed marked histopathological alterations, characterized by degeneration and loss of hippocampal neurons compared with the normal control group, indicating neuronal damage associated with STZ administration. Treatment with the combination of andrographolide and piperine resulted in noticeable restoration of hippocampal neuronal architecture, with a reduction in neuronal loss and improvement in the overall cellular arrangement. The restored neurons are indicated by arrows in Figure 11. Treatment with andrographolide alone also produced an apparent protective effect on hippocampal morphology, particularly at the higher dose of 60 mg/kg administered orally, where comparatively greater neuronal restoration was observed. These findings suggest that andrographolide, particularly at the higher dose, and its combination with piperine may attenuate the histopathological alterations induced by i.c.v.-STZ in the hippocampus.

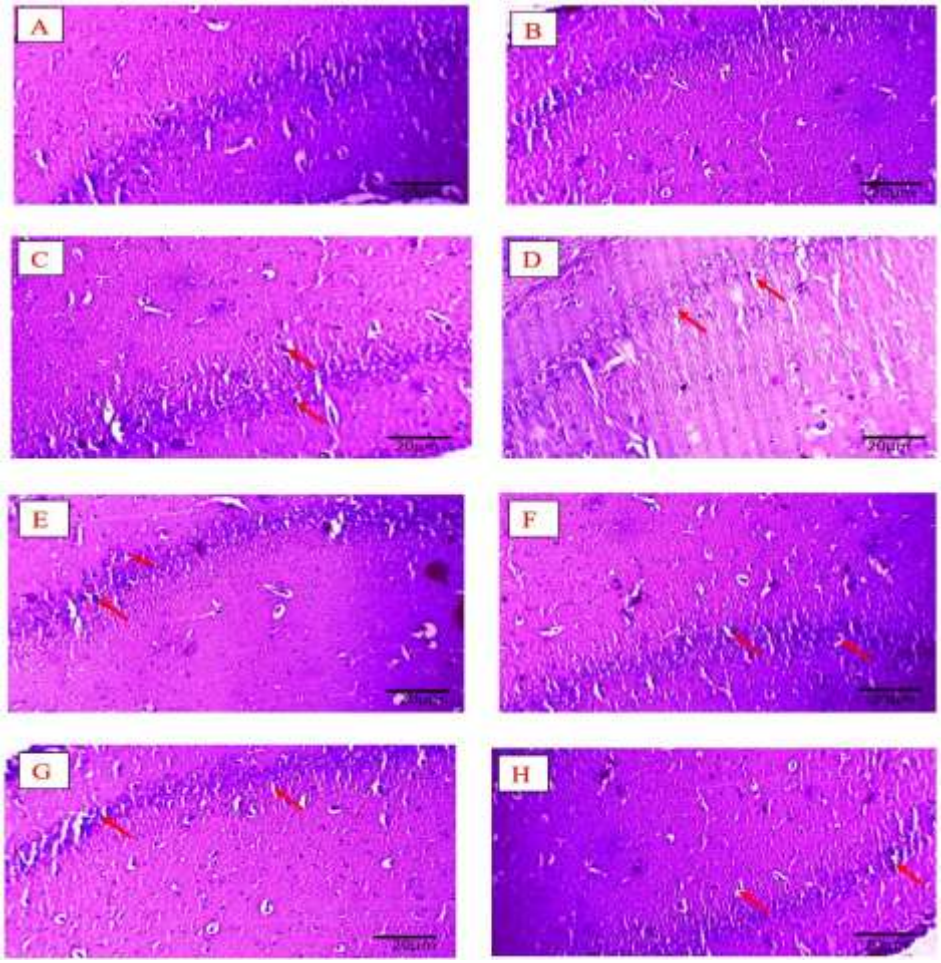


Figure 1: Effect of andrographolide and piperine treatment on histo-pathological deficits in streptozotocin-treated rats. A: Normal control; B: Piperine per se; C: ICV-STZ (3 mg/kg); D: STZ + ANDRO (15 mg/kg, orally); E: STZ + ANDRO (30 mg/kg, orally); F: STZ + ANDRO (60 mg/kg, orally); G: STZ + ANDRO (15 mg/kg) + piperine (2.5 mg/kg); H: STZ + donepezil (5 mg/kg, orally). (ICV-STZ: i.c.v.-streptozotocin; ANDRO: andrographolide; PIP: piperine)

Conclusion

The histopathological evaluation provided supportive structural evidence for the neuroprotective effects of andrographolide, both alone and in combination with piperine, against intracerebroventricular streptozotocin (ICV-STZ)-induced Alzheimer's disease-like neurodegeneration in adult Wistar rats. The hippocampus, a brain region critically involved in learning and memory, exhibited prominent histopathological alterations following ICV-STZ administration. Compared with the normal control group, the ICV-STZ group showed evident degeneration and loss of hippocampal neurons, indicating substantial neuronal injury associated with STZ-induced central neurotoxicity. These structural alterations were consistent with the behavioural and biochemical abnormalities observed in the experimental model and further supported the successful induction of AD-like neurodegenerative changes. Treatment with andrographolide resulted in a noticeable attenuation of the hippocampal neuronal damage induced by ICV-STZ. The protective effect was more evident at the higher dose of andrographolide (60 mg/kg, orally), where comparatively greater preservation and restoration of hippocampal neurons were observed. This dose-related improvement suggests that andrographolide may exert a protective influence on neuronal integrity, potentially through its previously reported antioxidant, anti-

inflammatory, and anti-apoptotic properties. By reducing oxidative and inflammatory insults, andrographolide may help preserve neuronal architecture and limit the progressive cellular damage associated with ICV-STZ-induced neurodegeneration. Importantly, administration of the andrographolide–piperine combination produced a more pronounced improvement in hippocampal histoarchitecture than andrographolide treatment alone. The combination group demonstrated appreciable restoration of neuronal organisation and reduced neuronal loss, with the preserved/restored neurons clearly evident in the histological sections. The enhanced structural protection observed with the combination may be attributed, at least in part, to the bioenhancing properties of piperine, which may increase the systemic availability and pharmacological exposure of andrographolide. In addition, the intrinsic antioxidant and anti-inflammatory properties of piperine may contribute to the overall neuroprotective response.

Taken together, the histopathological findings indicate that andrographolide can attenuate ICV-STZ-induced hippocampal neuronal degeneration and that its neuroprotective effect may be enhanced when administered in combination with piperine. The preservation of hippocampal neuronal architecture provides morphological support for the improvements observed in behavioural, oxidative, cholinergic, inflammatory, and neurochemical parameters. Thus, the andrographolide–piperine combination represents a promising experimental approach for mitigating neuronal damage associated with AD-like pathology. However, these findings are based on an experimental animal model and should be interpreted as preclinical evidence. Further studies involving quantitative histopathological scoring, molecular investigations, pharmacokinetic evaluation, and additional mechanistic studies are warranted to clarify the precise pathways responsible for the enhanced neuroprotective effect of the andrographolide–piperine combination and to establish its potential for further therapeutic development.

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Declarations

Ethical approval

Animals are approved by IAEC under protocol number ISFCP/IAEC/CPCSEA/Meeting No. 29 /2025/ Protocol No.431, ISF College of Pharmacy, Moga (Punjab)

Author contributions

Royal Patel authored the manuscript, organised the data, produced the figures, and performed the study.

Pradeep Kumar Mohanty conceived and developed the initial idea and concept for the paper and finalised the text.

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Competing interests

All authors declared the absence of any financial or personal conflicts about this work.

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