Nootropic and neuroprotective effects of ethanol extract of *Vateria indica* L bark on scopolamine-induced cognitive deficit in mice

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Original Research Article

Abstract

**Purpose:** To investigate the neuroprotective and memory-boosting properties of ethanol extract of *Vateria indica* bark on scopolamine-mediated defects in learning and memory in young mice.

**Methods:** The ethanol extract of *V. indica* bark was prepared via Soxhlet extraction and subjected to qualitative and quantitative phytochemical assessment. The acute toxicity of the extract was also evaluated in mice. Six groups of 3-month-old Swiss albino mice (6 per group) were used: normal control, negative control, piracetam group, 250 mg/kg *V. indica* extract alone group, 500 mg/kg *V. indica* extract alone group, 250 mg/kg *V. indica* extract + scopolamine group and 500 mg/kg *V. indica* extract + scopolamine group. The mice were pretreated with piracetam (standard nootropic drug) or varied doses of the extract for 14 days prior to induction of amnesia. With the exception of normal control group, amnesia induction using scopolamine (3 mg/kg) i.p. on day 14 at 1½ h after the last extract dose. Mice in normal and negative control groups received 0.5 % tragacanth orally at a dose of 10 mL/kg. Cognitive deficit was assessed using elevated plus maze (EPM), step-down avoidance, and Morris water maze (MWM) tests.

**Results:** Qualitative phytochemical screening of *V. indica* bark extract showed flavonoids, phenolics, glycosides, tannins, carbohydrate, saponins and steroids. The total phenol and total flavonoid contents were 580.96 ± 0.95 mg GAE/g extract and 66.89 ± 0.56 mg RE/g extract, respectively. The mice tolerated the extract up to 5000 mg/kg bwt. They all survived during and after the acute toxicity study and no significant changes in appearance or general behavior were noticed. The extract significantly enhanced learning and memory, and improved spatial recognition in scopolamine-induced amnesic mice (p < 0.05). Acetylcholinesterase (AChE) activity and levels of dopamine and noradrenaline were markedly higher in negative control mice than in normal control, but were significantly reduced after pretreatment with ethanol extract of *V. indica* bark (p < 0.05). The results of histopathological examination provided evidence in support of the protective effect of the extract on hippocampal and cortical neurons.

**Conclusion:** Pretreatment with ethanol extract of *V. indica* bark confers neuroprotection and enhances memory in young amnesic mice. Therefore, the extract of the plant can potentially be developed for the management of degenerative brain conditions.

**Keywords:** Alzheimer’s disease, Cognitive deficit, Neuroprotection, Phytochemicals, Vateria indica
INTRODUCTION

Alzheimer's disease (AD) is an irreversible neurodegeneration that impairs cognitive function such as learning, memory, intelligence, thinking and reasoning [1]. It has been reported that decreases in the levels of acetylcholine and free radicals in the brain contribute significantly to memory loss [2].

Brain neurotransmitters such as dopamine, noradrenaline and serotonin have received huge attention for their possible involvement in memory processes [3]. Declines in cognitive function may be due to generation of ROS, as well as changes in the levels of acetylcholine and the monoamines [4].

At present, nootropic drugs like piracetam and cholinesterase blockers like donepezil and rivastigmine are used to treat AD patients. However, their clinical use is limited by some drawbacks [5]. This has necessitated the search for novel plant-derived compounds that are safe and effective against the disease. Plants that have shown potential in this regard include Rosmarinus officinalis, Celastrus paniculatus, Nardostachys jatamansi, Ginkgo biloba, Ficus carica, Withania somnifera and Curcuma longa [6]. Studies have shown that the extracts of these plants can effectively modulate brain aging and prevent cognitive decline associated with AD.

Vateria indica Linn is a large evergreen tree that belongs to Dipterocarpaceae family. Its extracts possess anti-inflammatory, anthelmintic, anti-ulcer, antitumor and anticancer properties [7]. The plant extract is rich in phenols and flavonoids, thereby making it a potential neuroprotective agent, since polyphenolics have been reported to be neuroprotective [8].

This study investigated the neuroprotective and memory-enhancing effects of ethanol extract of V. indica bark on scopolamine-induced memory deficit.

EXPERIMENTAL

Sample collection

The stem bark of V. indica was collected from Dharwad forest, Karnataka, India, and identified by Dr. Rajesh Shastri of the Department of Pharmacognosy, Soniya Education Trust's (SET's) College of Pharmacy, Dharwad. Herbarium specimen was prepared using standard method and voucher number issued (SETCPD/Ph.cog/Herb/04/2017).

Preparation of extract

The plant material was washed, sliced to small sections, subjected to shade-drying, pulverized, and extracted with absolute ethanol for 12 h at 50 °C using a Soxhlet apparatus. The extract was concentrated using rotary evaporator and made into powder by lyophilisation.

The resultant dried extract was desiccated and its yield in terms of dried plant material was calculated. A suspension of the extract was thereafter prepared in 0.5 % gum tragacanth using mortar and pestle and administered orally to mice at varied doses.

Phytochemical screening

The extract was screened for the presence of different phytochemicals using standard methods [9]. High-performance thin-layer chromatography (HPTLC) fingerprinting of the ethanol extract was performed according to standard procedures [10]. Quantitative estimation of total flavonoid and total phenol were also carried out using standard procedures [11].

Experimental mice

Three-month-old Swiss albino mice weighing 22 – 28 g (mean weight = 25.00 ± 3.00 g) were used for this study. They were housed in metal cages under standard conditions and were freely given standard feed and water. The mice were exposed to light and dark photoperiod, and maintained at 25 °C and 48 % humidity. They were allowed one-week acclimatization period before commencement of the study.

The study received approval from the Ethical Committee of Najran University (no. 05-08-18EC) and the study procedures were carried out according to the guidelines of the National Institute of Health (NIH) for the use and care of experimental animals [12].

Acute toxicity study

This was carried out on the ethanol extract of V. indica bark in line with OECD method. Mice (n = 42) were fasted overnight prior to dosing. Varied doses of the extract (5 - 5000 mg/kg bwt) were administered orally using a gavage. Each mouse was observed during the first 24 h after dosing, for signs of toxicity, and thereafter observed every day for 14 days. The toxicity parameters monitored included sedation, convulsions, hypothermia, grooming, hyperactivity and mortality.
Experimental design for mice model of AD

Seven groups of three-month-old Swiss albino mice (6 per group 6) were used: normal control, negative control, piracetam group, 250 mg/kg bwt V. indica extract alone group, 500 mg/kg bwt V. indica extract alone group, 250 mg/kg bwt V. indica extract + scopolamine group and 500 mg/kg bwt V. indica extract + scopolamine group. The mice were pretreated with piracetam (standard nootropic drug) or varied doses of the extract for fourteen days before induction of amnesia. With the exception of normal control group, amnesia induction was done using scopolamine (3 mg/kg, i.p). [13]. Mice in normal and negative control groups received 0.5 % tragacanth orally at a dose of 10 mL/kg. The mice were thereafter subjected to behavioral model training sessions using EPM, MWM and passive shock avoidance apparatus for 14 days. On the 15th day, retention memory was recorded.

Evaluation of cognitive function

Elevated plus maze (EPM) test

The EPM test is a behavioral procedure used to assess short-term memory in animals. The apparatus comprised 2 closed and 2 open arms, centrally mounted on a base, and raised 25 cm above the floor. Acquisition trial was performed to measure transfer latency time on the 14th day of treatment, by placing each mouse at the end of an open arm, with its back towards the central platform. Transfer latency was defined as the time (sec) required by a mouse to move with all its four legs from the open arm into any closed arm. Each mouse was given a 2-min freedom of movement in the EPM before being transferred back to the cage. On the next day, retention trial was conducted to ascertain how well the mice have retained this learned task (memory). A significant decrease in transfer latency time was taken as improvement in memory [13].

Step-down-type passive shock avoidance test

Step-down behavior model was applied to measure long-term memory based on electric shock. The apparatus for step-down avoidance test comprises an illuminated box (27 × 27 × 27 cm), consisting of three wooden walls and a plexiglas wall. The grid floor was made of stainless steel rods (3 mm) and supplied with electric shock (20 V AC). A base made of wood was placed centrally on floor of the grid. The mice were individually placed in the center of the grid floor on the wooden platform. Electric shock was delivered for 5 sec immediately the mouse came down with four paws on the grid floor. The time required for the mouse to come down from the wooden platform onto the grid floor with all four paws was taken as the step-down latency. The step-down latency was recorded during the acquisition trial for each mouse. Retention trial was conducted after 24 h under similar conditions, except that the grid floor was free of electric shocks [14].

Morris water maze test (MWMT)

Spatial learning was determined with MWMT apparatus at a water temperature of 25 ± 2 °C. In one of the equally divided quadrants, a platform was positioned 2 cm under the water. The period spent to locate the platform (escape latency) was noted during each acquisition trial session. Acquisition training included two trial sessions at intervals of 3 - 4 h per day, for 1 week. After completion of acquisition training, MWMT was conducted on days 6, 10 and 14 of treatment. On location of the platform, a mouse was allowed to stay on it for another 10 sec. Any mouse that failed to locate the platform within 2 min was guided to it and allowed there for another 10 sec. A probe trial was conducted on day 7 by removing the concealed platform from the water pool. The period spent in target quadrant from where the platform was removed was noted [15].

Determination of AChE

Brain AChE activity was estimated using Ellman's procedure. The mice brains were isolated after cervical dislocation and immediately put in chilled saline prior to preparation of 10 % (w/v) homogenate in phosphate buffer, pH 8.0 (10 % w/v). The assay mixture contained exactly 0.4 mL of brain supernatant and 100 μL of 2-nitro benzoic acid, to which 2.6 mL phosphate buffer was added and thoroughly mixed, and absorbance was read at 412 nm. This was recorded as basal reading. The substrate acetylthiocholine (20 μL) was subsequently added, and absorbance change was then measured at 2-min intervals for 10 min [16]. Acetylcholinesterase activity was calculated as shown in Eq 1.

\[ R = (5.74 \times 10^{-4}) A/OC \quad \ldots \ldots \ldots \ldots \ldots \ldots (1) \]

where \( R \) = moles of substrate hydrolyzed/min/g brain tissue; \( A \) = absorbance change/min; and \( OC \) = tissue concentration (mg/mL).

Determination of levels of dopamine and noradrenaline in brain tissue

Brain tissues isolated from the mice after sacrifice were weighed and homogenized using
10 mL HCl-butanol, and brain levels of dopamine and noradrenaline were measured using the method of Schlumpf et al [17].

**Histopathological examination of brain hippocampus**

Excised brain tissues were fixed in 10 % neutral-buffered formal saline and processed for light microscopy according to standard methods, and examined under light microscope. Different hippocampal regions were selected and the neurons in CA1 region were counted with the help of morphometric lens in 0.25 mm² microscopic field, and the mean was calculated [18]. The hippocampus region was studied for neuronal damage, karyorrhexis, pyknotic black neurons, and number of dead neurons [19].

**Statistical analysis**

Data are expressed as mean ± SEM. Statistical analysis was performed using GraphPad Prism (5.0). Groups were compared using Tukey’s test. Statistical significance was assumed at $p < 0.05$.

**RESULTS**

**Phytochemical profile of extract**

Qualitative phytochemical screening of ethanol extract of *V. indica* bark revealed the presence of flavonoids, phenolics, glycosides, tannins, carbohydrate, saponins and steroids. The total phenol and total flavonoid contents were 580.96 ± 0.95 mg GAE/g extract and 66.89 ± 0.56 mg RE/g extract, respectively, while HPTLC fingerprinting of the extract revealed five prominent spots with their respective retention factors (Rf), indicative of the presence of active phytochemicals in the extract (Figure 1).

![Figure 1: Photo-documentation of the extract under UV light, depicting five prominent spots](image)

**Acute toxicity**

The mice tolerated the extract up to 5000 mg/kg. They all survived during and after the test, and no significant changes in appearance or general behavior were noticed.

**Effect of ethanol extract of *V. indica* bark on cognition**

As shown in Table 1, scopolamine significantly increased the transfer latency of the mice on days 14 and 15, when compared with normal control group ($p < 0.05$). However, pretreatment with piracetam or graded doses of ethanol extract of *V. indica* bark for 14 days significantly improved mice memory, relative to negative control mice ($p < 0.05$). Transfer latency was significantly lower in piracetam- and extract-treated mice than in scopolamine alone-exposed mice ($p < 0.05$). The scopolamine alone-exposed mice (negative control) showed markedly lower step-down latency when compared with normal control mice during acquisition and retention trials conducted on days 14 and 15, respectively ($p < 0.05$). Mice in the extract alone-treated groups showed significant improvement in learning and memory ($p < 0.05$). Scopolamine-induced memory deficit was significantly reversed after pretreatment with piracetam or extract ($p < 0.05$).

**Effect of ethanol extract of *V. indica* bark on spatial learning**

During the three consecutive training days (days 6, 10 and 14), the normal control group mice were able to locate the hidden platform more quickly than the negative control group. Scopolamine-treated mice exhibited significant delay in escape latency time, relative to normal control on days 10 and 14, respectively ($p < 0.05$). However, pretreatment with the extract significantly and dose-dependently reduced the escape latency time relative to negative control group ($p < 0.05$). Similarly, piracetam-treated mice showed significant improvement in spatial learning on days 10 and 14, when compared with negative control group ($p < 0.05$). During probe trial on day 15, mice in negative control showed significant reduction in time spent in target quadrant, when compared with normal control mice ($p < 0.05$). The extract- and piracetam-treated mice showed marked increase in time spent in target quadrant, relative to negative control mice ($p < 0.05$; Table 2).
Table 1: Transfer latency of mice as determined using EPM and step-down latency in passive shock avoidance tests

| Group                        | Latency in elevated plus maze (s) | Latency in passive shock avoidance (s) |
|------------------------------|-----------------------------------|----------------------------------------|
|                             | 14th day                          | 15th day                               |
| Normal control               | 29.00 ± 0.80                      | 32.50 ± 1.10                           |
| Negative control             | 55.50 ± 0.90                      | 56.00 ± 1.60                           |
| Piracetam                    | 34.30 ± 1.10                      | 32.50 ± 0.90                           |
| Extract alone (250 mg/kg)    | 49.10 ± 1.40                      | 47.30 ± 1.30                           |
| Extract alone (500 mg/kg)    | 38.50 ± 0.90                      | 37.60 ± 1.70                           |
| Extract (250 mg/kg) +        | 50.50 ± 0.90                      | 48.30 ± 1.50                           |
| scopolamine (3 mg/kg, i.p.)  | 38.00 ± 1.30                      | 36.60 ± 2.40                           |
| Extract (500 mg/kg) +        | 49.10 ± 1.40                      | 47.30 ± 1.30                           |
| scopolamine (3 mg/kg, i.p.)  | 38.50 ± 0.90                      | 37.60 ± 1.70                           |

Results are mean ± SEM; $^a p < 0.05$, relative to normal control; $^b p < 0.05$, vs negative control

Table 2: Effect of *V. indica* extract on scopolamine-induced learning and memory deficit

| Group                        | 6 (sec) | Day 10 (sec) | 14 (sec) | 15 (Probe trial) |
|------------------------------|---------|--------------|----------|------------------|
| Normal control               | 48.50 ± 4.00 | 24.50 ± 2.20 | 21.60 ± 1.80 | 79.80 ± 4.00 |
| Negative control             | 60.10 ± 3.60 | 55.10 ± 2.40 | 55.00 ± 2.50 | 46.00 ± 4.10 |
| Piracetam                    | 50.10 ± 2.80 | 33.00 ± 3.40 | 31.50 ± 2.10 | 152.70 ± 4.70 |
| Extract alone (250 mg/kg)    | 55.10 ± 3.60 | 41.30 ± 3.00 | 39.30 ± 3.40 | 71.10 ± 4.50 |
| Extract alone (500 mg/kg)    | 48.80 ± 3.00 | 34.80 ± 2.70 | 31.50 ± 3.10 | 133.00 ± 4.50 |
| Extract (250 mg/kg) +        | 50.10 ± 2.50 | 40.80 ± 2.30 | 41.00 ± 2.60 | 65.30 ± 3.80 |
| scopolamine (3 mg/kg, i.p.)  | 43.10 ± 4.30 | 38.30 ± 2.30 | 35.80 ± 2.80 | 126.20 ± 3.40 |

Results are mean ± SEM. $^a p < 0.05$, relative to normal control; $^b p < 0.05$, vs negative control

Effect of ethanol extract of *V. indica* bark on brain acetylcholinesterase activity and levels of monoamines

Acetylcholinesterase activity and levels of dopamine and noradrenaline were markedly higher in negative control mice than in normal control mice, but were significantly reduced after pretreatment with ethanol extract of *V. indica* bark ($p < 0.05$). Pretreatment with piracetam produced similar effect ($p < 0.05$; Table 3).

Histopathological features of brain tissue

Histopathological examination of normal control mice hippocampi showed viable hippocampal cells with normal CA1 neurons and minimum degeneration of neurons (Figure 2 A). The hippocampal region of negative control mice revealed significant toxicity with only a few number of viable neurons and significant neuronal degeneration as suggested by the presence of neuronal nuclear pyknosis.

Table 3: Activity of acetylcholinesterase and levels of dopamine and noradrenaline in brains of amnesic mice pretreated with ethanol extract of *V. indica* bark

| Group                        | AChE (nM/mm) | Dopamine (nmoles/min/g of tissue) | Noradrenaline (nmoles/min/g of tissue) |
|------------------------------|--------------|----------------------------------|----------------------------------------|
| Normal control               | 22.80 ± 5.20 | 861.20 ± 8.00                    | 244.50 ± 7.80                          |
| Negative control             | 13.70 ± 3.00 | 978.20 ± 5.40                    | 282.80 ± 7.50                          |
| Piracetam                    | 28.30 ± 1.90 | 887.20 ± 7.40                    | 251.00 ± 7.00                          |
| Extract alone (250 mg/kg)    | 24.60 ± 2.90 | 936.50 ± 6.60                    | 267.30 ± 8.40                          |
| Extract alone (500 mg/kg)    | 23.40 ± 4.20 | 898.50 ± 10.80                   | 257.80 ± 7.70                          |
| Extract (250 mg/kg) +        | 31.50 ± 3.40 | 941.80 ± 9.70                    | 276.00 ± 4.70                          |
| scopolamine (3 mg/kg, i.p.)  | 33.70 ± 5.20 | 906.80 ± 6.70                    | 262.20 ± 8.00                          |

Data are mean ± SEM. $^a p < 0.05$, relative to normal control; $^b p < 0.05$, vs negative control. For dopamine and noradrenaline, data are fluorescent excitation height (nmoles/min/g of tissue). AChE = acetylcholinesterase
eosinophilic neuronal necrosis and significant reduction in CA1 neurons (Figure 2 B). The results of histopathological examination of the hippocampi of mice in piracetam group showed excellent protection against scopolamine-induced toxicity as revealed by less neuronal necrosis and increased number of CA1 neurons (Figure 2 C). Pretreatment with ethanol extract of V. indica bark without scopolamine challenge resulted in normal viable neurons with less pyknotic cells (Figures 2D and E). Similarly, pretreatment with 250 mg/kg ethanol extract of V. indica bark accompanied by scopolamine challenge produced moderate damage to neuronal cells and increased CA1 neuron density (Figure 2 F), while 500 mg/kg of the extract produced mild neuronal degeneration and increases in the number of CA1 neurons (Figure 2 G and Figure 3).

**DISCUSSION**

Scopolamine has been reported to cause cholinergic deficit via the promotion of oxidative stress and increased cholinesterase activity. Cholinergic deficit adversely affects short- and long-term memory, as well as learning acquisition.

Extracts of V. indica have exhibited anti-inflammatory, anthelmintic, anti-ulcer, antitumor and anticancer effects [7]. The plant extract is rich in phenols and flavonoids, thereby making it a potential neuroprotective agent, since polyphenolics have been reported to be neuroprotective [8]. The neuroprotective properties of ethanol extract of V. indica bark on scopolamine-mediated memory loss in mice were determined in this study.

In this study, there were significant reductions in transfer latency in mice treated with graded doses of ethanol extract of V. indica bark, relative to negative control mice, an indication that the extract may be nootropic and could confer neuroprotection against scopolamine-induced amnesia in mice. Pretreatment with piracetam (standard nootropic drug) also produced significant reduction in transfer latency. Ethanol extract of V. indica bark and piracetam treatment significantly increased the step-down latency on day 15 after training, when compared with negative control group, suggesting improvement in memory. It is likely that scopolamine caused memory impairment in the mice by forming cholinergic blemishes in their brain neurons. Ethanol extract of V. indica bark may contain active principles that are able to reverse scopolamine-induced cholinergic dysfunction. The extract significantly and dose-dependently enhanced the spatial memory of the mice. These results suggest that ethanol extract of V. indica bark stimulated spatial learning and memory retention ability of mice, and are in agreement with results obtained in previous studies [20].

To elucidate the neuroprotective mechanisms of V. indica extract, biochemical parameters such as acetylcholinesterase activity, whole brain noradrenaline and dopamine levels were evaluated. It has been reported that increases in acetylcholinesterase activity lead to cholinergic deficit due to increased degradation of acetylcholine. Scopolamine administration in animals potentiates acetylcholinesterase activity, leading to central cholinergic dysfunction [20]. The present study revealed that acetylcholinesterase activity and levels of dopamine and noradrenaline were higher in negative control group than in normal control group, but were significantly decreased after pretreatment with ethanol extract of V. indica bark. Pretreatment with piracetam produced similar effect. The extract may have effectively
mitigated scopolamine-induced amnesia in mice. These findings are consistent with those of previous reports [21]. Thus, the nootropic and neuroprotective effects of ethanol extract of V. indica bark may be attributed to augmentation of cholinergic pathways in the hippocampus.

Interactions between complex neurotransmitter systems in the brain play a key role in memory and learning. A large body of evidence indicates the involvement of catecholamines, particularly noradrenaline and dopamine in cognitive processes [3]. Studies have shown that optimum levels of excitatory neurotransmitters such as noradrenaline, dopamine and 5-hydroxytryptophan (5-HT) are essential for normal memory function [22]. Under normal laboratory condition, monoamines have been shown to improve memory at moderate doses, but promote memory deficit at higher doses. Hence, elevated levels of noradrenaline and dopamine in the brain are linked to dementia and memory impairment as seen in AD [23]. Pronounced decreases in concentrations of these amines can precipitate other disease conditions such as Parkinson’s disease and psychosis. The results obtained in this study suggest that the nootropic effect of ethanol extract of V. indica bark may be associated with central catecholaminergic modulatory action, and are in agreement with those of previous studies [6,21].

Antioxidants play vital roles in the management of neurodegenerative disorders by rescuing the neurons from free radical-induced oxidative damage [13,24]. Free radicals are highly reactive species which cause oxidative damage to healthy cells, leading to disease conditions such as neoplasms, diabetes mellitus and AD [2]. Natural antioxidants like flavonoids, polyphenols and stilbenes, have great free radical scavenging potential [25]. Scopolamine has been reported to cause oxidative stress by increasing TBARS and decreasing GSH levels in animal models of amnesia [13].

Phytochemical investigation of ethanol extract of V. indica bark revealed the presence of flavonoids, glycosides, polyphenolics, tannins, carbohydrate, saponins and steroids. Scientific evidence abound on the neuroprotective effect of polyphenolic compounds and their potential to promote learning, memory and cognitive functions. The antitumor, anti-inflammatory, anthelmintic, anti-ulcer and anticancer properties of V. indica are attributed to the presence of phytochemicals such as phytosterols, tannins, bergenin, resveratrol tetramers, hopeaphenol, isohopeaphenol, vateriaaphenol B, vateriaphenol C, vaticanol B, vaticanol C, ε-Viniferin and epicatechin [7]. Similarly, in this study, the neuroprotective and nootropic effects of ethanol extract of V. indica bark could be attributed to these phytochemicals.

The hippocampus and prefrontal cortex are involved in spatial learning and cognition. Scopolamine-induced memory deficit was found to be associated with significant reduction in CA1 neurons, presence of neuronal nuclear pyknosis and eosinophilic neuronal necrosis. Pretreatment with ethanol extract of V. indica bark and piracetam for 14 days effectively ameliorated scopolamine-induced neuronal degeneration and death. These results are suggestive that V. indica has great promise as a novel and effective nootropic and neuroprotective agent.

CONCLUSION
Pretreatment with ethanol extract of V. indica bark confers neuroprotection and enhances memory in young amnesic mice. Thus, it may be helpful in the management of neurodegenerative diseases.

DECLARATIONS

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Conflict of interest
No conflict of interest is associated with this work.

Contribution of authors
We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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