Neuroprotective effect of *Hydrocotyle sibthorpioides* against monosodium glutamate-induced excitotoxicity

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**ABSTRACT**

We aimed to evaluate the neuroprotective effect of *H. sibthorpioides* against monosodium-glutamate (MSG) induced excitoneurotoxicity in rats. We randomly divided the animals into 11 groups (\(n = 8\)) and subjected them to high doses of MSG (2 g/kg body weight) and the test dose (1 week). The test chemicals were *H. sibthorpioides* extracts of petroleum ether, chloroform, methanol, and water. We used Dizocilpine-hydrogen-maleate as a standard and assessed the cognitive property using Morris-water-maze and elevated-plus-maze. After the experimental period, we evaluated the biochemical parameters. We found chloroform and methanolic extracts significantly enhanced the cognitive behaviour of rats compared to control. Biochemical analysis suggested that there was a high level of antioxidants and lower levels of glutamate and proinflammatory cytokines in the cortex and hippocampus. We concluded that chloroform and methanolic extracts of *H. sibthorpioides* enhanced the level of antioxidants, decreased proinflammatory-cytokines and glutamate in the brain, and thus prevented the monosodium-glutamate-induced-excite-neurotoxicity.

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1. Introduction

Monosodium glutamate (MSG) is a flavoring agent and many countries use it without any restrictions (Farombi and Onyema 2006). Hence, people consume large doses of MSG that lead to an increase in blood and brain glutamate concentration (Shivasharan et al. 2013). A high brain MSG concentration increases excitability and activates the proteolytic enzymes to cause severe toxicity (Weil et al. 2008). This neurotoxicity implicates neurodegenerative diseases, which can produce symptoms like dizziness, flushing, numbness, sweating, and increased oxidative stress (Farombi and Onyema 2006; Ambrosi et al. 2014). High doses of MSG also increase the oxygen free radicals and damage the neurons of the hypothalamic region (Meldrum 1993; 2000; Farombi and Onyema 2006). Hence, i.p. injection of MSG in high dose will be a successful model for the study of oxidative stress and excitotoxic neuronal damage in the rat brain.

People of Assam use *Hydrocotyle sibthorpioides* Lam. (Family: Araliaceae) traditionally as a brain tonic (Hazarika et al. 2019; 2021). Report also suggests a high level of antioxidants in *H. sibthorpioides* (Huang et al. 2008). Earlier, we demonstrated the neuroprotective effect of *H. sibthorpioides* extracts on aluminum chloride induced neurotoxicity in rats (Hazarika et al. 2022). However, no study investigated the neuroprotective effect of *H. sibthorpioides* extracts against glutamate-induced excitotoxicity. Therefore, we aimed to evaluate the neuroprotective activity of *H. sibthorpioides* extracts against MSG-induced neurotoxicity in rats.

2. Results and discussion

The extracts of *H. sibthorpioides* showed the presence of many secondary metabolites (Table S2). Methanolic extract of *H. sibthorpioides* (MEHS) showed maximum % yield, phenolic content, flavonoids content and asiaticoside (Table S3, Figure S1). Chloroform extract (CEHS) showed significantly high asiaticoside.

Morris’s water maze is considered to be a very good model for the study of spatial memory (Vorhees and Williams 2006) and elevated plus maze (EPM) for cognition. In
EPM, as compared to control initial transfer latency (%ITL, 0.99 ± 0.06) was significantly high in CEHS (200 mg/kg, %ITL, 1.05 ± 0.07; 100 mg/kg, %ITL, 1.08 ± 0.06; \( p < 0.001 \)), MEHS (200 mg/kg, %ITL, 1.08 ± 0.06; 100 mg/kg, %ITL, 1.08 ± 0.07; \( p < 0.001 \)) and dizocilpine hydrogen maleate (DHM, 0.05 mg/kg; %ITL, 1.09 ± 0.05) (Figure S2a). CEHS, MEHS and DHM showed significant decrease \( (p < 0.001) \) in time to reach the hidden platform, searching frequency and time in the target area compared to control in Morris’s water maze.

Cortex and hippocampus are most susceptible to oxidative stress damage because these areas are highly enriched in nonheme iron that is involved in the catalysis of ROS production (Hill and Switzer 1984; Venkataraman et al. 2007; Tanamatayarat et al. 2012). Treatment with DHM, CEHS, and MEHS significantly altered their level (Figure S3).

GABA reduced significantly in the cortex and hippocampus in groups treated only with MSG (Figure S4). MEHS reversed the MSG induced GABA depletion from the cortex and hippocampus. On the other hand, the level of glutamate significantly increased \( (p < 0.001) \) in the group treated with the only MSG when compared to the control. However, the level of glutamate reduced significantly \( (p < 0.001) \) in groups treated with CEHS and MEHS when compared to groups treated only with MSG (Figure S4).

The glutamate exposure is also responsible for the increased production of proinflammatory cytokines such as IL-6, IL-\( \beta \), and TNF-\( \alpha \) (Chaparro-Huerta et al. 2005). Our results are per the above finding that the group treated only with MSG significantly increased the pro-inflammatory cytokines; however, administration of CEHS and MEHS significantly reduced \( (p < 0.001) \) the level of proinflammatory cytokines suggesting that the extracts possess anti-inflammatory properties (Figure S5).

The present study also evidenced the loss of neuronal structure, necrosis in the cortex and hippocampal region of rats treated only with MSG. However, the MSG caused damage was less in groups treated with DHM, CEHS, and PEHS, showing a protective effect towards neurodegeneration (Figure S6). The protective effect of *H. sibthorpioides* may be attributed to the free radical scavenging property and proinflammatory cytokines inhibiting property.

### 3. Conclusions

Chloroform and methanolic extracts of *H. sibthorpioides* inhibit inflammatory cytokines and scavenge oxygen free radicals to show its neuroprotective effect against MSG-induced neurotoxicity. However, it requires further investigations to reveal the specific mechanism.

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