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Screening of Anticonvulsant Profile of *Habenaria Intermedia* Tubers in Rats Using Well Established Model

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ABSTRACT

A review of the literature found that *Habenaria intermedia* has not had any systematic pharmacological research done to support the traditional claims made for the majority of its neuropharmacological activities, particularly for its anticonvulsant profile. Therefore, it was thought to be beneficial to test *H. intermedia* tubers anticonvulsant profile. The hydroalcoholic extract and its ethyl acetate fraction of plant tubers were prepared using reference procedures and showing presence of alkaloids, flavonoids, coumarins and tannins as major classes of compounds. The two parametres were accessed such as mean onset of action (sec) and mortality (%) in anticonvulsant activity screening and results are described in table 15 and figure 2 following the administration of hydroalcoholic extract (100, 200, or 400 mg/kg, p.o.), ethyl acetate fraction (25 or 50 mg/kg, p.o.), diazepam (2 mg/kg, p.o.), and the control (vehicle, p.o.). The experimental protocol was divided into three groups such as control, standard and test. Each group of animals received pentylenetetrazole (80 mg/kg, i.p.) before 45-60 min prior takes control, standard and test drugs. The hydroalcoholic extract and its ethyl acetate fraction of *Habenaria intermedia* tubers were exhibit anticonvulsant action in a dose dependent manner. The hydroalcoholic extract (400 mg/kg) and its ethyl acetate fraction (50 mg/kg) of *Habenaria intermedia* tubers were exhibit anticonvulsant action statistically equivalent to standard drug. On the basis of literature survey available on pure natural products neuropharmacological activities, it can be suggested that anticolvulsant activity of *Habenaria intermedia* tubers are attributed to these natural classes of compounds.

Key words: *Habenaria intermedia*, anticonvulsant, pentylenetetrazole, flavonoids, phenols.

Introduction

Mainly found in India (Himalayas, Himachal Pradesh, Kashmir, Uttarakhand, and Sikkim), Pakistan, Nepal, and Bhutan is *Habenaria intermedia* D. Don (Riddhi; family: Orchidaceae) (Balkrishna et al., 2012). Traditional medicine has made extensive use of *Habenaria intermedia* D. Don to treat a variety of illnesses. It serves as a tonic for health. It is a component of the Astavarga formulation, which consists of eight rejuvenating medications. Additionally, it goes into making "Chyawanprash," a popular Ayurvedic tonic. According to Khare (2007), Kirtikar and Basu (1994), Warrier et al. (1994), its tubers are used as an emollient (having a soothing/cooling effect), an aphrodisiac, a depurative (removing impurities from the body), an anthelmintic, a nervine, and a cardiac tonic. They are also used to treat leprosy, burning sensations, asthma, fever, cold, insanity, and other skin diseases (Prajapati et al., 2003). According to Jagetia et al. (2004), it has antioxidant action. Additionally, it is utilized in the production of "Salep," a tuber-based flour (Ahuja, 2003). Following a review of the literature, it was discovered that H. intermedia includes flavonoids, tannins, phenol (gallic acid) (Goudar et al., 2015; Habbu et al., 2012), coumarin (scopoletin) (Habbu et al., 2012; Reynolds and Wilson, 1991), and alkaloids (Rajashekhar et al., 2015). Starch and minerals are also present. A review of the literature showed that the immunomodulatory (Sahu et al., 2013), antistress (Habbu et al., 2012), and hepatoprotective (Goudar et al., 2015) properties *of H. intermedia* have all been thoroughly studied. A study of the literature revealed that the majority of the conventional claims made for *H. intermedia* neuropharmacological activities—particularly for its anticonvulsant profile—have not been supported by systematic pharmacological research. Testing the anticonvulsant profile of *H. intermedia* tubers was therefore deemed advantageous.

MATERIALS AND METHODS

Plant material

Habenaria intermedia tubers were procured from Himalaya Herbs Store, Madhav Nagar, Saharanpur, (Uttar Pradesh), India in January, 2024. The identity of plant was confirmed from online literature related to their microscopic characters.

Preparation of test samples and their chemical testing

Using Soxhlet apparatus technology, the hydroalcoholic extract from dried plant material was made after defating with n-hexane solvent. Additionally, reflux technology was used to generate the ethyl acetate fraction from the hydroalcoholic extract (Kumar and Kumar, 2015; Prakash et al., 2015). To

determine if phytomolecules were present or absent, a chemical screening process was applied to both the hydroalcoholic extract and its ethyl acetate fraction (Farnsworth, 1966).

Anticonvulsant activity

Animals

Male SD rats of body weight 250-300 g, procured from Akal College of Pharmacy and Technical Education, Mastuana Sahib, Sangrur were used for anticonvulsant activity using using pentylenetetrazole seizure model (Vogel, 2002). The animals were fed with normal laboratory pellet diet and water *ad libitum*. The approval was taken from Institutional Animal Ethics Committee of Akal College of Pharmacy and Technical Education, Mastuana Sahib, Sangrur before carrying out animal studies (ATRC/30/23, dated 26/12/2023).

Vehicle and standard drugs

Distilled water + Tween 80 (2%) was used as vehicle for preparing various test doses of crude extract and fraction in such a concentration as to administer a volume ranging 1-2 ml to the rats. Phenytoin sodium injection was used as a standard anticonvulsant drug at the dose of 20 mg/kg, *i.p.* Pentylenetetrazole was used to induce convulsions at the dose of 80 mg/kg, *i.p.*

Experimental protocol

Experimental protocol comprising groups 1-7 was designed to assess anticonvulsant activity of various crude extract / fractions of plant tubers.

Group 1 - Control group received pentylenetetrazole (80 mg/kg, *i.p.*).

Group 2 - Standard group received phenytoin (20 mg/kg, *i.p.*) + pentylenetetrazole (80 mg/kg, *i.p.*).

Groups 3 - Test groups received 100 mg/kg doses of hydroalcoholic extract + pentylenetetrazole (80 mg/kg, *i.p.*).

Groups 4 - Test groups received 200 mg/kg doses of hydroalcoholic extract + pentylenetetrazole (80 mg/kg, *i.p.*).

Groups 5 - Test groups received 400 mg/kg doses of hydroalcoholic extract + pentylenetetrazole (80 mg/kg, *i.p.*).

Groups 6 - Test groups received 25 mg/kg doses of ethyl acetate fraction + pentylenetetrazole (80 mg/kg, *i.p.*).

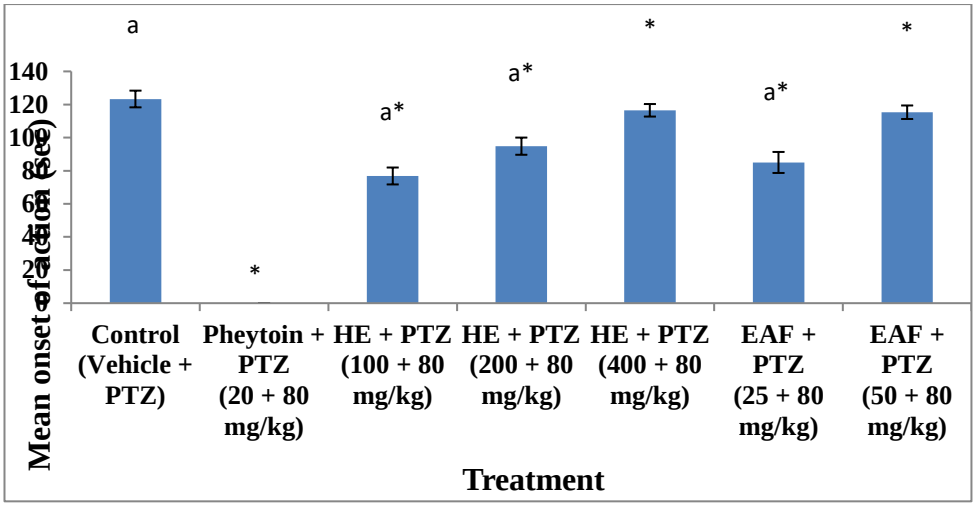
Groups 7 - Test groups received 50 mg/kg doses of ethyl acetate fraction + pentylenetetrazole (80 mg/kg, *i.p.*).

Statistics

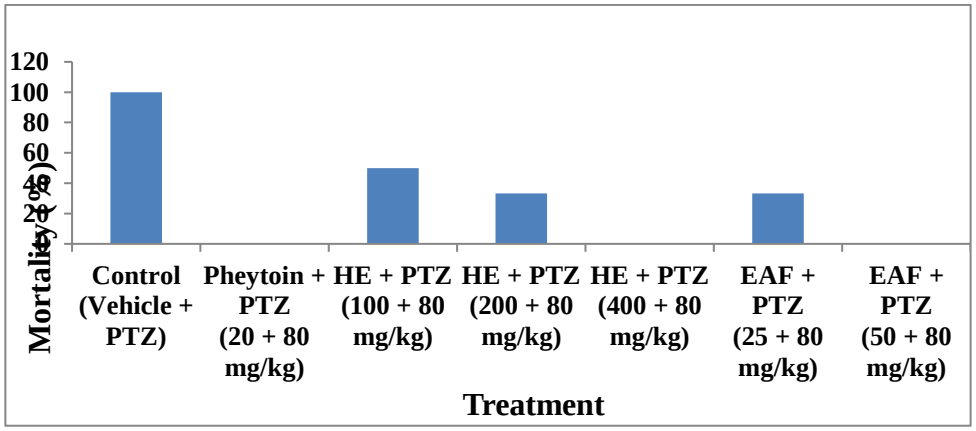
The results were expressed as mean ± standard deviation (S.D.). The anticonvulsant activity of test drugs was compared with that of standard drug and control by one way analysis of variance (ANOVA) followed by Student-Newman-Keul's test (Scheffer, 1980).

RESULTS AND DISCUSSION

The ethyl acetate fraction (in relation to hydroalcoholic extract) and the hydroalcoholic extract's percentage yield (w/w) were found to be 5.90 and 20.80 % w/w respectively. The main purpose of extraction of plant material with n-hexnae is to remove various greasy materials from plant to avoid any interference for further extraction and pharmacological profiles. The hydroalcoholic extract of plant tubers showing presence of alkaloids, flavonoids, coumarins, proteins, tannins and carbohydrates. Further, the hydroalcoholic extract was purified to generate ethyl acetate fraction to remove (phytoconstituent rich fraction) to remove highly polar substances which are not responsible for pharmacological activities. The phytochemical testing of ethyl acetate fraction showing presence of alkaloids, flavonoids, coumarins and tannins. The hydroalcoholic extract and its ethyl acetate fraction of *Habenaria intermedia* tubers were investigated for anticonvulsant activity screening using well reported model in scientific databases named using pentylenetetrazole induced seizure model. The two parametres were accessed such as mean onset of action (sec) and mortality (%) in anticonvulsant activity screening and results are described in figure 1 following the administration of hydroalcoholic extract (100, 200, or 400 mg/kg, p.o.), ethyl acetate fraction (25 or 50 mg/kg, p.o.), diazepam (2 mg/kg, p.o.), and the control (vehicle, p.o.). The experimental protocol was divided into three groups such as control, standard and test. Each group of animals received pentylenetetrazole (80 mg/kg, i.p.) before 45-60 min prior takes control, standard and test drugs. The hydroalcoholic extract and its ethyl acetate fraction of *Habenaria intermedia* tubers were exhibit anticonvulsant action in a dose dependent manner. The hydroalcoholic extract (400 mg/kg) and its ethyl acetate fraction (50 mg/kg) of *Habenaria intermedia* tubers were exhibit anticonvulsant action statistically equivalent to standard drug. Other doses hydroalcoholic extract (100 or 200 mg/kg) and its ethyl acetate fraction (25 mg/kg) of *Habenaria intermedia* tubers were exhibit anticonvulsant action but statistically not equivalent to standard drug.



(A)



(B)

Figure 1: Anticonvulsant activity of *Habenaria intermedia* tubers using pentylenetetrazole induced seizure model. HE, Hydroalcoholic extract; EAF, Ethyl acetate fraction.

n=6; The data is expressed as Mean ± S.D.; **P<0.05* vs. Control; [a]*P< 0.05* vs. Standard; one way ANOVA followed by Student Newman Keul's test.

Conclusion

According to Kumar et al. (2014), bioactive extract and fractions of *Habenaria intermedia* tubers may have anticonvulsant properties by altering excitatory and inhibitory neurotransmission through effects on voltage-gated ion channels, GABA(A) receptors, and glutamate-mediated excitatory neurotransmission. Additionally, potentiating GABA neurotransmission may be achieved by inhibiting calcineurin (protein phosphatase 2B), reducing activation of NMDA extracsynaptic receptors, and preventing the activation of extracellular regulated kinase and p38α mitogen activated protein kinase pathways.

According to the literature that is currently accessible, coumarins, alkaloids, and flavonoids are essential for the treatment of neurological illnesses (Mittal et al., 2016). There are several plant reports wherein flavonoids have demonstrated central nervous system (CNS) activities. These include the flavonoids hesperidin (Marder et al., 2003), gossypin (Duraisami et al., 2009), diosmin (Fernandez et al., 2006), naringin (Fernandez et al., 2009), and quercetrin (Kang et al., 2010); alkaloids hypaconitine (Nesterova et al., 2010), montanine (Silva et al., 2006), neferine (Sugimoto et al., 2008), pseudo-akuammigine (Duwiejua et al., 2002), and yohimbine (Chermat et al., 1997); coumarins esculetin (Wang et al., 2012), imperatorin (Luszcki et al., 2009), osthole (Luszcki et al., 2009), and marmesinin (Kang and Kim 2007). Our findings imply that these naturally occurring groups of chemicals are responsible for the anticolvulsant activity of *Habenaria intermedia* tubers, which is consistent with recent observations.

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