PROTECTIVE ACTIVITY OF HAB-E-JUND A UNANI FORMULATION AGAINST CONVULSIONS IN MICE

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Summary

Unani system of medicine (Unanipathy) originated in Greece, based on the principles propounded by Galen, a Greek Practitioner and was called Galenic. After him many Arab and Persian scholars enriched the system and became Unani. Medicines in Unani are prepared from plants, animals and minerals. Hab-e-jund is a Unani medicine prescribed for febrile convulsions. The drug was tested against convulsions induced by maximum electroshock (MES) and pentylenetetrazol (PTZ). Oral administration of 100mg/kg and 300mg/kg of Hab-e-jund given as suspension in 3% gum acacia reduced duration of MES-induced hind limb extension reduced PTZ-induced myoclonic spasms and clonic convulsions in a dose dependent manner.

Key Words: Unani, Hab-e-jund, Epilepsy, Febrile Convulsions, Maximum electroshock, Pentylenetetrazol,

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Introduction

Unani system of medicine (Unanipathy) originated in Greece, based on the principles propounded by Galen, a Greek Practitioner and was called Galenic. After him many Arab and Persian scholars enriched the system and became Unani.[1] Some notable scholars of the science of Arab medicine are as follows: Al Tabbari (838–870AD), Al Razi (Rhazes) (846–930AD), Al Zahrawi (930–1013AD), Ibn Sina (Avicenna) (980–1037AD), Ibn Al Haitham (960–1040AD), Ibn Al Nafees (1213–1288Ad) and Ibn Khaldun (1332–1395AD).[2] Now it has become a part of Indian traditional system of medicine. Hab-e-Jund (HJ) is a Unani formulation prescribed for febrile convulsions. This study was undertaken as there is no scientifically tested and published literature available. It consists of Paeonia officinalis (12g), Delphinium denudatum (12g), Castoreum (12g), Pimpinella anisum (12g), Aloe barbidensis (12g), Wrightia tinctoria (12g), Ptychotis ajowan (12g) and trace amounts of Cow gall bladder stones, Musk.[3,4]

Paeonia officinalis is useful in cerebral infarction,[5] epilepsy,[6] cognitive disorders[7] and some hepatic disorders.[8] Delphinium denudatum has anticonvulsant,[9] antifungal[10] and Morphine de-addictive properties.[11] Castoreum is a dried and macerated castor sac scent glands from the male or female beaver (Castor canadensis) used extensively in perfume industries.[12] It is used in alternative medicine as antiseptic, antispasmodic, epilepsy, hysteria, asthma, muscular tremors and as emmenagogue.[13] Pimpinella anisum has antiulcer,[14] antioxidant, antimicrobial,[15] carminative, digestive, antiseptic, diuretic and useful in constipation and insomnia. In Unani it is used for dyspepsia, nausea, colic and epilepsy.[16] Aloe barbidensis has antioxidant,[17] wound healing,[18] bacteriostatic,[19] anticancer[20] and useful in skin disorders.[21] Wrightia tinctoria has antinociceptive,[22] *Wrightia tinctoria* (Roxb.) is useful in toothache,[23] as stomachic, antidiarrhoeal, antihemorrhagic,[24] antidandruff,[25] antiulcer[26] and in psoriasis.[27] Ptychotis ajowan (Synonym–Tachyspermum ammi) has anthelmintic,[28] antilithiatic,[29] antihyperlipidaemic[30] and antifungal activities.[31] Gall stones of cow (Cow Bezoar) is used in alternative medicines in febrile convulsions, fever, convulsions, stroke and other illnesses.[32-34] Musk is an odiferous secretion derived from the musk gland under the abdomen near the pubis of the male musk deer (*Moschus moschiferus* Linn). It is one of the most famous traditional Chinese medicines, and is used as a cardiac and general stimulant, an aphrodisiac, and an anti-spasmodic.[35] It is used in perfume industry, and also used as antiseptic, antihistaminic, antianginal, spasmolytic, central nervous system depressant, stimulant and antibacterial.[36,37]

Though the formulation, Hab-e-jund (HJ) is being used in Unani medicinal system for febrile convulsions, there is no scientific evidence available for the efficacy of the same. Hence this study was taken up to test its protective activity against convulsions induced by maximum electroshock (MES), pentylenetetrazole (PTZ) in mice. Drugs which increased brain contents of gamma amino butyric acid (GABA) have exhibited anticonvulsant activity against seizures induced by maximum electroshock (MES) and pentylenetetrazol (PTZ).[38] The MES is best-validated method for assessment of antiepileptic drugs in generalized tonic-clonic seizures.[39,40] Drugs useful in absence seizures suppress PTZ induced convulsions.[41,42]

Materials and Methods

Drugs

The formulation Hab-e-jund (HJ) was obtained as research sample from M/s Asian pharmacy, Shakar gunj, Hyderabad, India and was used as received. The formulation was fed by triturating and suspending in 3% gum acacia in water (vehicle).

The vehicle was used as control in the experiments. Pentylenetetrazol (Sigma, USA), Diazepam (Calmpose inj., Ranbaxy, India), Phenytoin (Epsolin inj., Cadila, India) were used in this study and were dissolved in water for injection and administered in a volume of 1ml/100g to mice.

Animals

Male Swiss albino mice weighing 20-28g were housed in groups of six under standard lab conditions (temperature $25^{o}\pm1^{o}$C, relative humidity 55±5%, 12h:12h dark:light cycle) with standard pellet food and water *ad libitum*. The animals were transferred to the laboratory at least 1h before the start of the experiment. The experiments were performed during the day (09:00-16:00h) and as per the guidelines of the Committee for the Purpose of Supervision and Control of Experiments on Animals (CPCSEA), Government of India. The Institutional Animal Ethics Committee approved the study protocol (IAEC/SUCP/01/2007).

Maximum electroshock (MES)-induced convulsions[42]

Mice were divided into 5 groups (n=6). Group I served as control and received only vehicle. Groups II, III and IV were treated orally with HJ at a dose of 30, 100, and 300mg/kg, respectively. Group V received 20mg/kg i.p., phenytoin. The mice received a current of 50mA for 0.2 sec duration through electroconvulsiometer (MKM, India) using ear electrodes after 60min of oral administration of HJ and 30min after i.p. administration of phenytoin. The incidence and duration of extensor tonus was noted. A complete abolition of hind limb tonic extension was considered as 100% protection.

Pentylenetetrazol (PTZ)-induced convulsions[42]

Five groups of mice (n=6) were treated with 80mg/kg s.c. PTZ, 60min after oral administration of either HJ (30, 100, and 300mg/kg) or vehicle or 30 min after i.p. administration of diazepam (4mg/kg). The animals were observed for 30min for onset, presence or absence of clonic convulsions and mortality.

Statistical Analysis

The data obtained were analyzed using one-way ANOVA and Kruskal-Wallis test followed by Dunn's test. $P<0.05$ was considered statistically significant.

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Results

MES-induced convulsions

The duration of tonic hind limb extension in mice treated with vehicle was 24.1 ± 4.2sec. HJ at 30mg/kg did not show significant anticonvulsant activity (Fig 1). Doses 100 and 300mg/kg significantly reduced the severity of seizures. The HJ doses 30, 100, 300 and phenytoin 20mg/kg exhibited hind limb extension for 23.2 ± 2.7, 7 ± 2.1, 4.8 ± 0.8, 3.3 ± 0.8sec respectively (Table 1).

PTZ-induced convulsions

In mice treated with vehicle, myoclonic spasm and clonic convulsions appeared after 97.5 ± 13.7 sec and 128 ± 7.2 sec respectively and all the control mice died after seizures. HJ significantly and dose dependently inhibited the onset and incidence of convulsions (Fig 2). The convulsions were completely abolished by the HJ 300mg/kg and Diazepam 4mg/kg (Table 2).

Table 1. Effect of HJ and Phenytoin against MES-induced convulsions.

Groups	Duration of hind limb Extension (Sec) Mean ± SD	Death (n=6)	P (Dunn's test)
Control	24.1 ± 4.2	5/6	
HJ 30mg/kg	23.2 ± 2.7	4/6	>0.05 ns
HJ 100mg/kg	7 ± 2.1	0/6	<0.05*
HJ 300mg/kg	4.8 ± 0.8	0/6	<0.001***
Phenytoin 20mg/kg	3.3 ± 0.8	0/6	<0.001***

The data were analyzed by Kruskal-Wallis test KW=25.115 (non parametric ANOVA, *P*=0.0002, from chi square distribution) followed by Dunn's multiple comparison test.
ns-Not significant *Significant ***Highly significant

Fig 1. Effect of HJ and Phenytoin on MES-induced convulsions in mice.

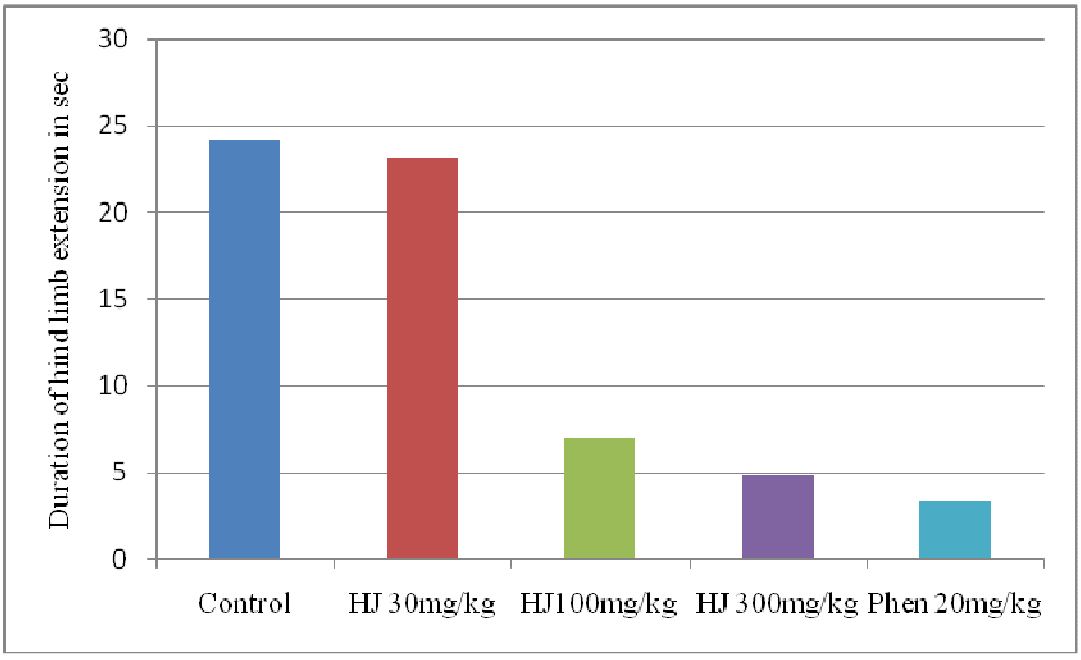
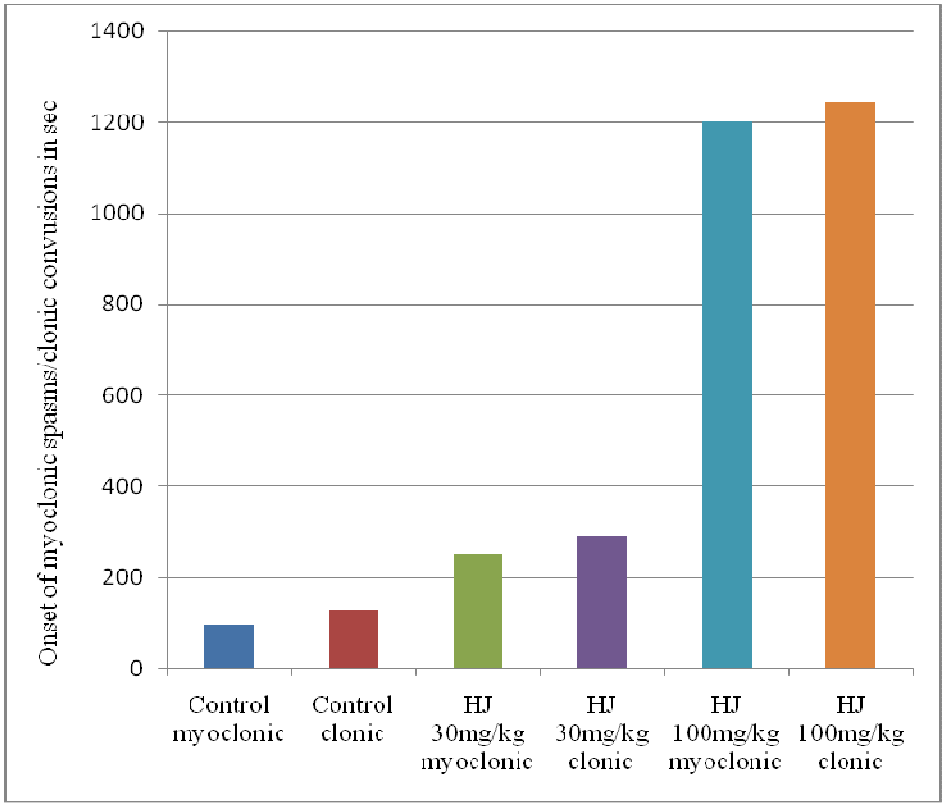


Table 2. Effect of HJ and Diazepam against PTZ-induced convulsions

Groups	Onset of myoclonic spasm (sec) Mean ± SEM	Onset of clonic convulsion (sec) Mean ± SEM	Death (n=6)
Control	97.5 ± 13.7	128 ± 7.2	6/6
HJ 30mg/kg	253.3 ± 15.1*	290 ± 15.8*	4/6
HJ 100mg/kg	1200 ± 118.5†	1243.3 ± 110.6†	0/6
HJ 300mg/kg	A	A	0/6
Diazepam 4mg/kg	A	A	0/6

The data were analyzed by Kruskal-Wallis test KW=15.205 (non parametric ANOVA, P=0.0005, from chi square distribution) followed by Dunn's multiple comparison test.
*P >0.05 (Not significant) $^{\dagger}P$ < 0.001 (Highly significant)
A- Absence of any seizure activity

Fig 2. Effect of HJ and Diazepam against PTZ-induced convulsions



*HJ 300mg/kg and Diazepam showed complete protection and no symptoms were seen

Discussion

The formulation HJ showed significant antiepileptic activity in both MES and PTZ- induced convulsions in a dose dependent manner. The results suggest that the anticonvulsant action of the formulation is mediated by Cl^- channel of the GABA/benzodiazepine receptor complex. At lower dose (30mg/kg) anticonvulsant activity was not seen, but 100mg/kg of the formulation significantly reduced seizure threshold and decreased the incidence of death. Since inhibition of MES-induced convulsions predicts activity against generalized tonic-clonic seizures (Grandmal epilepsy) and cortical-focal seizures[39,40] and inhibition of PTZ-induced convulsions against partial or absence seizures,[41] this study signifies the beneficial role of HJ in generalized tonic-clonic and absence seizures. HJ in Unani is prescribed only for febrile convulsions. The present study shows its beneficial effects even in generalized tonic-clonic and absence seizures.

Significant advances were made in recent years to treat epilepsy using second generation drugs. Polypharmacy is often advocated to 30% of epileptic patients for treating refractory partial or generalized seizures.[43] However none of the drugs completely control seizures.[44] Therefore despite the beneficial effects of currently available drugs, there is still a need for broadly acting anticonvulsant drugs possessing multiple mechanisms of action with decreased adverse effects, preferably originating from natural products.

Conclusions

The results of the present study show the benefits of Hab-e-jund and could be of particular interest in generalized tonic-clonic seizures controlled only by toxic doses of anticonvulsant drugs used currently. Hence further research is necessary to determine the benefits in other forms of epilepsies and studies for its drug interactions.

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