IN VITRO ANTIOXIDANT ACTIVITY OF HAB-E-JUND [UNANI MEDICINE] PRESCRIBED FOR FEBRILE CONVULSIONS

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Summary

Unani system of medicine (Unanipathy) originated in Greece, enriched by Persians and Arabs and now became an integral part of Alternative medicinal systems of India. Hab-e-jund is a Unani medicine prescribed for febrile convulsions. The drug was tested for antioxidant activity, as there is growing evidence of role of free radicals in disease progression in epilepsy and benefits of concomitant antioxidant administration. Currently available antiepileptic drugs either exacerbate or decrease free radicals. Hab-e-jund was tested for free radical scavenging and metal chelating activity. It showed considerable in vitro antioxidant activity in a dose dependent manner.

Key Words: Unani, Hab-e-jund, Ascorbic acid, Disodium ethylene diamine tetra acetic acid, Free radical scavenging, Metal chelation

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Introduction

Unani system is one of the traditional alternative medicinal systems of India. Hab-e-jund (HJ) is a Unani medicine prescribed for febrile convulsions. 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 and Musk.[1,2] Our earlier study showed its activity against maximal electroshock and pentylenetetrazole induced convulsions in mice.[3] Epilepsies are common and frequently devastating disorders and current therapy involves – limiting sustained repetitive firing of neurons by promoting inactive state of voltage activated Na^+ channels (carbamazepine, phenytoin, valproate, topiramate, zonisamide), enhancing GABA–mediated synaptic inhibition (benzodiazepines, barbiturates, vigabatrin, valproate, tiagabine) and some reduce the flow of Ca^{2+} through T- type Ca^{2+} channels (valproate, ethosuximide).[4]

Recent studies showed the significance of oxidative stress, mitochondrial dysfunction and free radicals in epilepsy.[5,6] Oxidative stress is caused by – reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS are either free radicals (superoxide anion radical $^1O_2$•, •OH) or non radicals that are oxidizing agents and / or easily convert into radicals (HOCl, HOBr, O_3, $ONOO^-$, H_2O_2). Similarly RNS are either free radicals (NO•, NO_2•) or non radicals (HNO_2, N_2O_4). These are capable of damaging nucleic acids, lipids, proteins and carbohydrates and also can cause DNA damage, cellular damage and neuronal death.[7,8] Cells contain two types of natural defense systems – enzymes to detoxify (viz., superoxide dismutase, catalase and peroxidase) and antioxidants (vitamins C and E, glutathione, ferritin and uric acid). Saturation of these defense systems causes oxidative stress.[9] The defense mechanisms act by removing oxygen or decreasing local oxygen concentration, removing catalytic metal ions, ROS and RNS, quenching or scavenging initiating free radicals, breaking the chain of initiated sequence, enhancing endogenous antioxidant defenses by up-regulating expression of genes encoding the antioxidant enzymes, repairing oxidative damage caused by radicals, increasing elimination of damaged molecules and not repairing excessively damaged molecules in order to minimize introduction of mutation.[10]

Thus therapies aimed at reducing oxidative stress may ameliorate tissue damage and favorably alter the clinical course.[11] Several Studies showed that the current antiepileptic drugs either ameliorate or exacerbate oxidative stress and free radical mediated tissue injury.[12-16]

In the present study we have taken up two in vitro antioxidant models – free radical scavenging activity and metal chelating activity, to test the antioxidant potential of HJ.

Materials and Methods

Drug

The formulation Hab-e-jund (HJ) was obtained as research sample from M/s Asian pharmacy, Shakar gunj, Hyderabad, India and was triturated, dissolved in methanol and filtered.

Chemicals

DPPH• (1,1-Diphenyl-2-picrylhydrazyl radical) was obtained from Sigma, New Delhi, India. L-Ascorbic acid (AA), ferrous chloride ($FeCl_2$), ferrozine, Disodium ethylene diamine tetra acetate (EDTA) and solvents were obtained from sd-fine Chemicals, Mumbai, India. All the chemicals were of analytical grade.

Free Radical Scavenging Activity

The free radical scavenging activity of HJ was measured employing the method of Blois.[17] To 1ml of different concentrations (1,10,20,30,40,50,60,70,80,90,100 µg/ml) of HJ, 1ml of 0.1mM solution of DPPH• in methanol was added and stirred vigorously on vortex mixer. The reaction mixture was kept in dark for 30minutes and the absorbance was measured at 517nm using UV-Spectrophotometer (Shimadzu, Japan). A control containing only DPPH• was taken and different concentrations of AA (1-100µg/ml) as standard. All measurements were made in triplicate and their means taken. Percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \left\{(A_o - A_s)/A_o\right\} \text{x } 100$$

Where A_o and A_s are absorbencies of control and sample/standard respectively. IC_{50} values introduced by Brand-Williams et al[18] were calculated by plotting % Inhibition vs Concentration.

Metal Chelating Activity

The chelation of ferrous ions was estimated by method of Dinis et al.[19] To the tubes containing 1.7ml of deionized water, 50µl of 0.2mM $FeCl_2.4H_2O$ and 50µl of different concentrations of HJ (20,40,60,80,100 µg/ml) were added mixed and kept aside for 1min. The reaction was initiated by the addition of 0.2ml of 5mM ferrozine, mixed on a vortex mixer and after 10min the absorbance of the solutions were measured at 562nm in a UV-Vis Spectrophotometer (Shimadzu, Japan). All tests and analyses were made in triplicates. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated as follows

$$\% \text{ Inhibition} = \left\{(A_o - A_s)/A_o\right\} \text{ x } 100$$

Where A_o and A_s are absorbencies of control and sample/standard respectively.

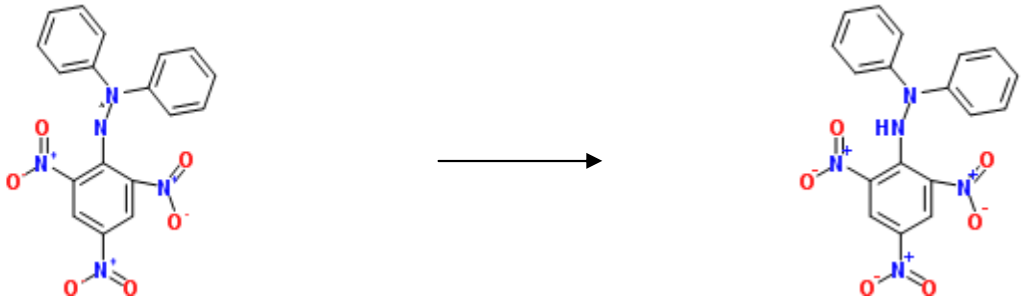
Statistical Analysis

Results were presented as mean ± SD. Statistical analysis were performed using Graphpad instat software. The values $p < 0.05$ were considered significant after performing Duncan's multiple range test.

Results And Discussion

Free Radical Scavenging Activity

DPPH• is considered to be a model of lipophilic radicals which initiate lipid auto oxidation. DPPH• is characterized as a stable free radical by virtue of delocalization of the spare electron over the molecule as a whole so that the molecules do not dimerize, as would be case with other free radicals. The delocalization also gives rise to deep violet color, characterized by an absorption band at 517nm. When a solution of DPPH• is mixed with a substance that can donate hydrogen atom, it reduces to DPPHH (1,1-Diphenyl-2-picrylhydrazine, pale yellow color from the picryl group still present).[20]



DPPH• (517nm)
(1,1-diphenyl-2-picrylhydrazyl radical)

DPPHH
(1,1-diphenyl-2-picrylhydrazine)

The free radical scavenging activity of HJ was evaluated through its ability to quench the DPPH• using ascorbic acid as reference. The results are shown in Table 1. HJ showed free radical scavenging activity (Fig 1) and the IC_{50} values of HJ and AA were found to be 83 ± 6.6µg/ml and 20.3 ± 2.3 µg/ml respectively (Fig 2).

Table 1. DPPH• Scavenging activity of AA and HJ

Conc. µg/ml	AA %Inhibition		HJ % Inhibition	
	Mean	SD	Mean	SD
0	0	0	0	0
1	8.8	9.2	6	2.1
10	24.1	4.7	7.1	2.5
20	49.1	5.6	7.5	1.8
30	64.9	1.7	7.6	4.8
40	86.1	0.5	15.4	5.5
50	95.6	0.2	16.9	3.1
60	95.7	0.1	17.7	8.2
70	96.9	0.2	29.4	15.0
80	97.2	0.2	39	13.5
90	97.2	0.1	61.6	9.6
100	97.3	0.2	69.7	3.4

$p<0.05$ Duncan's multiple range test

Fig 1. DPPH Scavenging Activity [% Inhibition of DPPH Radical] By AA and HJ

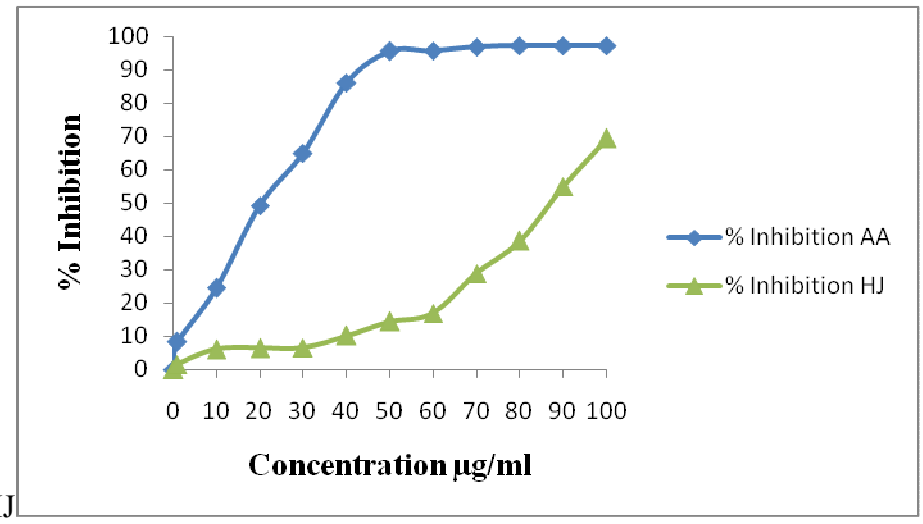
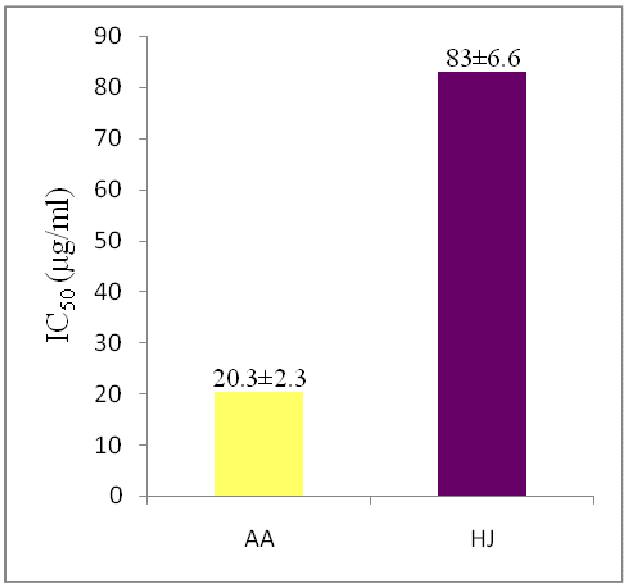
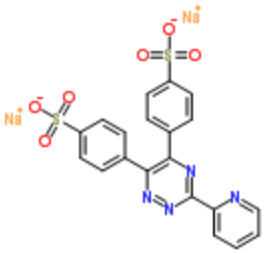


Fig 2. IC_{50} (µg/ml) of AA and HJ against DPPH free radical



Metal Chelating Activity

Metal chelating activity is claimed as one of the antioxidant mechanisms, since it reduces the concentration of bivalent transition metal ions which act as catalysts in lipid peroxidation leading to formation of hydroxyl radicals and hydrogen peroxide decomposition reactions via fenton reactions.[21] Ferrozine quantitatively forms complexes with Fe^{2+}, however in presence of chelating agents, the complex formation is disrupted and the dark red color (562nm) of the complex decreases. The color reduction allows the estimation of chelating activity of co-existing chelator.



Ferrozine
(3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine)

HJ interfered with chelation of Fe^{2+} ions in a dose dependant manner reducing the intensity of the color of Fe^{2+} - ferrozine complex. Results are shown in Table 2 and Fig 3. Concentration for Inhibition 50% of metal chelation by HJ was found to be 60 ± 6.2 µg/ml and that of disodium EDTA was 10.7 ± 0.6 µg/ml (Fig 4).

Table 2. Fe^{2+} Chelating Activity of EDTA and HJ

Conc µg/ml	EDTA %Inhibition		HJ % Inhibition	
	Mean	SD	Mean	SD
0	0	0	0	0
20	98.5	0.4	9	0.4
40	98.6	0.3	34.8	6.8
60	98.7	0.2	48.4	4.7
80	98.9	0.1	59.6	2.1
100	99	0.1	67.9	1.6

$p<0.05$ Duncan's multiple range test

Fig 3. Fe^{2+} Chelating Activity of EDTA and HJ

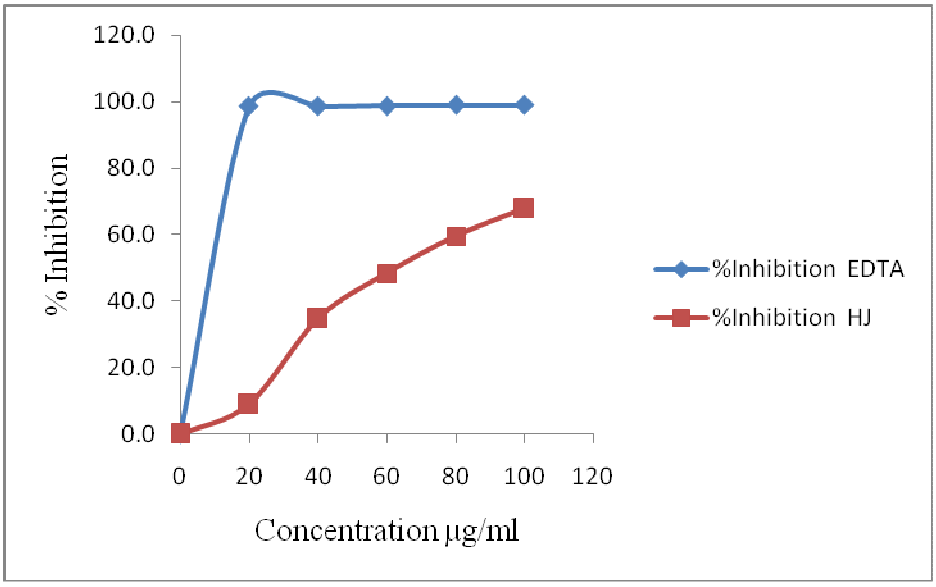
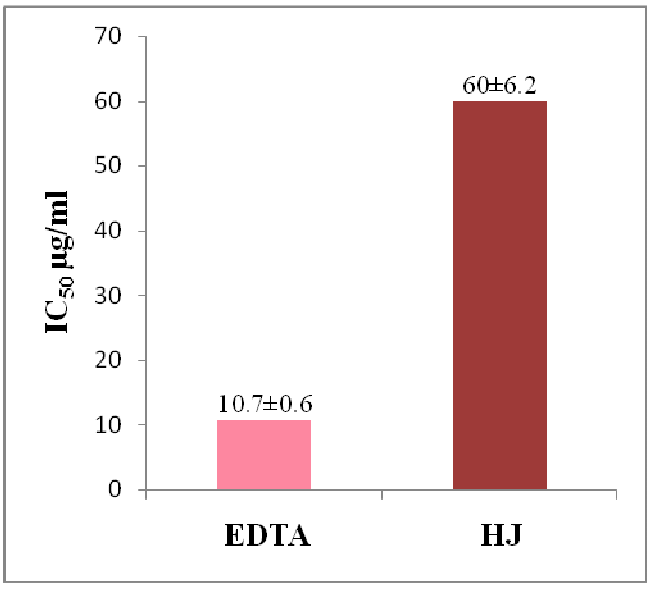


Fig 4. Inhibition of metal chelation by EDTA and HJ



Conclusions

Unani medicines are used extensively, but lack scientific evidence. Hab-e-jund showed Considerable antiepileptic[3] and antioxidant activity. By its free radical scavenging and metal chelating activity, HJ besides antiepileptic activity, might reduce the free radical generation and quench the radicals already formed and inhibit neuronal damage. Further studies are required to determine its mechanism of action and toxicities.

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