Journal of Pharmacy and Alternative Medicine
ISSN 2222-5668 (Paper) ISSN 2222-4807 (Online)
Vol.2, 2012

Research Article

In Vitro Antioxidant Activity of Habbe Sara [Unani Medicine] Prescribed for Febrile Convulsions

Hafiz Muhammad Mazhar Asjad[1]*, Muhammad Shoaib Akhtar[1], Muhammad Asad[1], Badar Din[2], Faisal Gulzar1 and Fakhar ul Hassnain[1]

[1]Department of Pharmacology, Faculty of Pharmacy, University of Sargodha, Sargodha 40100, Pakistan
[2]Department of Chemistry, Faculty of Science, University of Sargodha, Sargodha 40100, Pakistan
* Corresponding author: asjad313@yahoo.com

Received: 20 October 2012
Revised: 18 November 2012
Accepted: 19 November 2012

Abstract

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

Keywords: Unani, Ascorbic acid, Disodium ethylene diamine tetra acetic acid, Free radical scavenging, Metal chelation

1. Introduction

Unani system is one of the traditional alternative medicinal systems of Pakistan and India. Habbe Sara is a Unani medicine prescribed for febrile convulsions. The test formulation, Habbe Sara is composed of extracts of herb/plants each 100 mg silver coated pill contains: Castoreum (Jund) 33.33 mg, Aloe vera (Dried Latex) (Aelwah Zard) 33.33 mg and Boswellia serrata (Kundur) 33.33 mg (Rehman, 1985 and Kabeeruddin, 1989). 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 Ca2+ through T- type Ca2+ channels (valproate, ethosuximide) and liver disorders are treated by hepatoprotective agents which have antioxidant activity (Anupama et al., 2009 and McNamara, 2006).

Recent studies showed the significance of oxidative stress, mitochondrial dysfunction and free

radicals in liver damage and epilepsy (Sudha, 2001 and Patel, 2002). Oxidative stress is caused by – reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS are either free radicals (superoxide anion radical 1O2•, •OH) or non radicals that are oxidizing agents and / or easily convert into radicals (HOCl, HOBr, O3, ONOO-, H2O2). Similarly RNS are either free radicals (NO•, NO2•) or non

radicals (HNO2, N2O4). These are capable of damaging nucleic acids, lipids, proteins and carbohydrates and also can cause DNA damage, cellular damage and neuronal death (Halliwell et al., 1999 and Gulcin et al., 2003). 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 (Arzimanoglou et al., 2002). 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 (Wood et al., 2006).

Thus therapies aimed at reducing oxidative stress may ameliorate tissue damage and favorably alter the clinical course (Costello and Delanty, 2004).Several Studies showed that the current antiepileptic drugs either ameliorate or exacerbate oxidative stress and free radical mediated tissue injury (Liu et al., 1998 and Solowiej et al., 2003)

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 Habbe Sara.

2. Materials and Methods

2.1. Drug

The formulation Habbe Sara was obtained as research sample from Madina pharmacy, Satellite Town, Sargodha, Pakistan and was triturated, dissolved in methanol and filtered.

2.2 Chemicals

DPPH• (1,1-Diphenyl-2-picrylhydrazyl radical) was obtained from Sigma. L-Ascorbic acid (AA), ferrous chloride (FeCl2), ferrozine, Disodium ethylene diamine tetra acetate (EDTA) and solvents were of analytical grade.

2.3 Free radical scavenging activity

The free radical scavenging activity of Habbe Sara was measured employing the method of Blois (Blois, 1958). To 1ml of different concentrations (1,10,20,30,40,50,60,70,80,90,100 µg/ml) of Habbe Sara, 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 UVSpectrophotometer (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:

$$\% \text{ Inhibition} = (Ao-As)/Ao \times 100$$

Where Ao and As are absorbencies of control and sample/standard respectively. IC50 values introduced by Brand-Williams et al were calculated by plotting % Inhibition vs Concentration (Williams et al., 1995).

2.4 Metal chelating activity

The chelation of ferrous ions was estimated by method of Dinis et al (Dinis et al., 1994). To the tubes containing 1.7ml of deionized water, 50µl of 0.2mM FeCl2.4H2O and 50µl of different concentrations of Habbe Sara (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-Fe2+ complex formation was calculated as follows

$$\% \text{ Inhibition} = (Ao-As)/Ao \times 100$$

Where Ao and As are absorbencies of control and sample/standard respectively.

2.5 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.

3. Results and Discussion

3.1 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) (Molyneux, 2004).

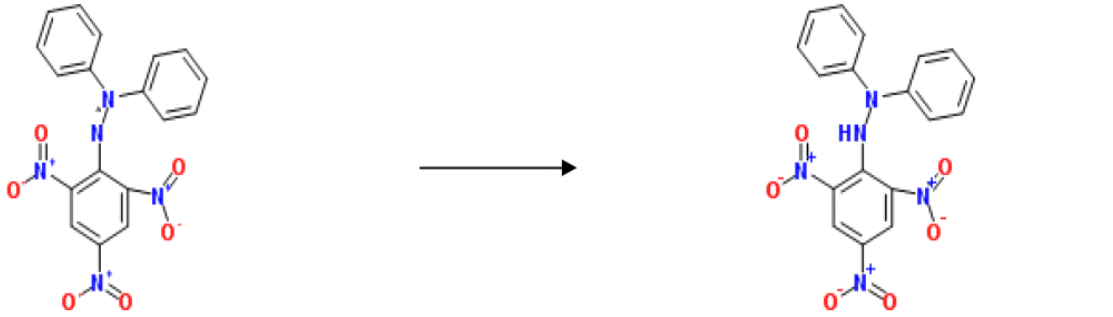


Figure 1. Reduction of DPPH•

The free radical scavenging activity of Habbe Sara was evaluated through its ability to quench the DPPH• using ascorbic acid as reference. The results are shown in Table 1. Habbe Sara showed free radical scavenging activity and the IC50 values of Habbe Sara and AA were found to be 83 ± 6.6µg/ml and 20.3 ± 2.3 µg/ml respectively (Fig 3).

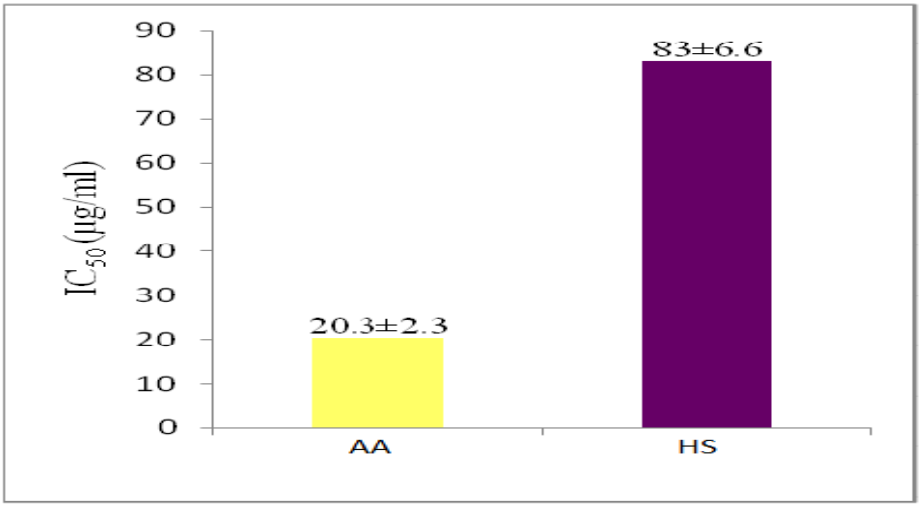


Fig3. IC50 (µg/ml) of AA and HS against DPPH free radical

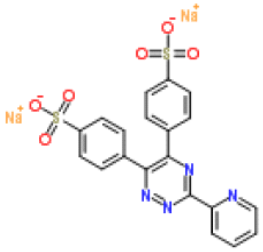
Table 1. DPPH• Scavenging activity of AA and HS

Conc. µg/ml	AA %Inhibition		Habbe Sara % Inhibition	
	Mean	±SD	Mean	±SD
0	0.0	0.0	0.0	0.0
1	8.5	8.9	5.8	3.1
10	23.6	4.5	14.7	5.2
20	48.2	5.3	36.2	5.8
30	65.9	1.5	65.3	4.7
40	87.2	0.5	80.3	4.6
50	96.1	0.2	95.2	2.1
60	96.2	0.1	96.1	2.0
70	96.8	0.2	95.2	2.1
80	97.1	0.2	96.9	1.0
90	97.1	0.1	96.9	0.8
100	97.2	0.2	98.8	0.8

$p<0.05$ Duncan's multiple range test

3.2 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 (Halliwell, 1997). Ferrozine (Fig 4) quantitatively forms complexes with Fe2+, 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) Habbe Sara interfered with chelation of Fe2+ ions in a dose dependant manner reducing the intensity of the color of Fe2+-ferrozine complex. Results are shown in Table 2 and Fig 5.



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

Fig 4. Structure of ferrozine

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

Conc. µg/ml	EDTA %Inhibition		Habbe Sara % Inhibition	
	Mean	±SD	Mean	±SD
0	0	0	0	0
20	98.5	0.4	9	0.4
40	98.4	0.3	32	6.8
60	98.6	0.2	50	4.7
80	98.7	0.1	58	2.1
100	99.1	0.1	68.5	1.6

$p<0.05$ Duncan's multiple range test

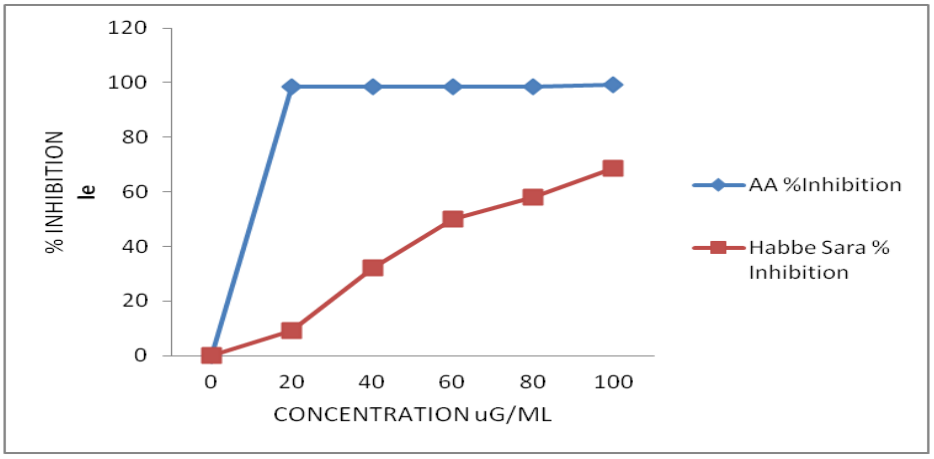


Fig 5. Fe2+ Chelating Activity of EDTA and HS

Concentration for Inhibition 50% of metal chelation by Habbe Sara was found to be 60 ± 6.2 µg/ml and that of disodium EDTA was 10.7 ± 0.6 µg/ml Fig 6.

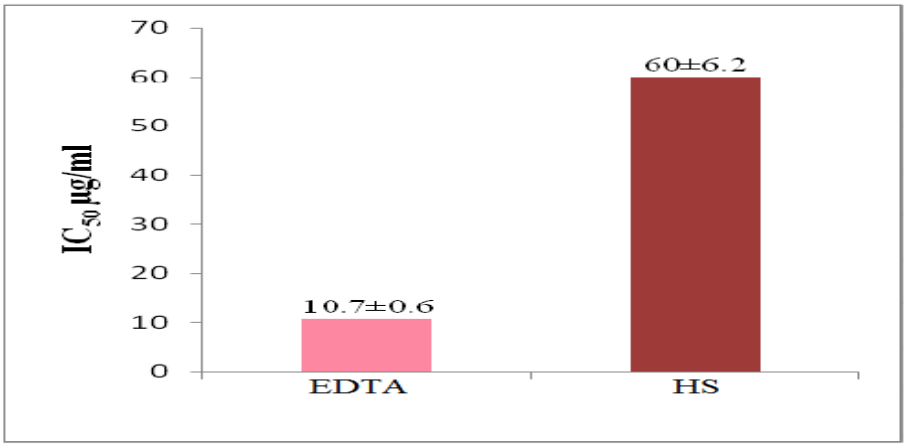


Fig 6. Inhibition of metal chelation by EDTA and HS

4. Conclusion

Unani medicines are used extensively, but lack scientific evidence. Habbe Sara showed considerable antiepileptic and antioxidant activity. By its free radical scavenging and metal chelating activity, Habbe Sara 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.

References

Anupama K, Satyanarayana S, Mukkanti K, Abedulla KKPavan K. (2009). Protective activity of Hab-E-Jund a Unani formulation against convulsions in mice. Pharmacologyonline. 3:724-731.

Arzimanoglou A, Hirsch E, Nehlig A, Castelnau P, Gressens P. DeVasconcelos AP. (2002). Epilepsy and Neuroprotection: An Illustrated Review. Epi dis. 4(3):173-182.

Blois MS. (1958). Antioxidant determinations by the use of a stable free radical. Nature. 181:1199-1200.

Brand-Williams W, Cuvelier ME, Berset C. (1995). Use of free radical method to evaluate antioxidant activity. Lebensm Wiss Technol. 28:25-30.

Cengiz M, Yuksel A, Seven M. (2000). The effects of carbamazepine and valproic acid on the erythrocyteglutathione, glutathione peroxidase, superoxide dismutase and serum lipid peroxidation in epileptic children. Pharmacol Res. 41:423-425

Costello DJ, Delanty N. (2004). Oxidative injury in epilepsy: potential for antioxidant therapy? Expert Rev Neurother. 4:541-553.

Dinis TCP, Madeira VMC, Almeida MLM. (1994). Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys. 315:161-169.

Gulcin I, Oktay M, Kirecci E, Kufrevioglu OI. (2003). Screening of antioxidant and antimicrobial activities of anise (Pimpinella anisum L.) seed extracts. Food Chem. 83: 371–382.

Halliwell B, Gutteridge JM. (1999). Free Radicals in biology and medicine. Oxford: Oxford University Press.

Halliwell B. (1997). Antioxidants: the basics- what they are and how to evaluate them. Adv Pharmacol. 38:3-20.

Kabeeruddin M. Biyaz-e-Kabeer. Hikmat Book Depot, Hyderabad: India.

Liu CS, Wu HM, Kao SH, Wei YH. (1998). Serum trace elements, glutathione, copper/zinc superoxide dismutase, and lipid peroxidation in epileptic patients with phenytoin or carbamazepine monotherapy. Clin Neuropharmacol. 21:62-64.

Maertens P, Dyken P, Graf W, Pippenger C, Chronister R, Shah A. (1995). Free radicals, anticonvulsants, and the neuronal ceroid-lipofuscinoses. Am J Med Genet. 57:225-228

McNamara JO. Pharmacotherapy of epilepsies In: Laurence L. Brunton, ed. Goodman and Gilman's. (2006) The Pharmacological Basis of Therapeutics, 11th ed. USA: McGraw Hill Medical Publishers : 501-525.

Molyneux P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol. 26(2):211-219.

Patel MN. (2002). Oxidative Stress, mitochondrial dysfunction and epilepsy. Free Radical Res. 36(11):1139-1146.

Rehman ZH. Kitabul Murakhabat. Publication division, Aligarh Muslim University, Madhya Pradesh: India.

Solowiej E, Sobaniec W. (2003). The effect of antiepileptic drug therapy on antioxidant enzyme activity

and serum lipid peroxidation in young patients with epilepsy. Neurol Neurochir Pol. 37:991-1003.

Sudha K, Rao AV, Rao A.(2001). Oxidative stress and antioxidants in epilepsy. Clin Chim Acta. 303(1):19-24.

Verrotti A, Basciani F, Trotta D, Pomilio MP, Morgese G, Chiarelli F. (2002). Serum copper, zinc, selenium, glutathione peroxidase and superoxide dismutase levels in epileptic children before and after 1 year of sodium valproate and carbamazepine therapy. Epilepsy Res. 48:71-75.

Wood LG, Gibson PG, Garg ML. (2006). A review of the methodology for assessing in vivo antioxidant capacity. J Sci Food Agri. 86:2057-2066.