WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 5, 1233-1241 Research Article ISSN 2278 – 4357

EVALUATION OF ANTIOXIDANT ACTIVITY IN VARIOUS MARKETED HEPATIC FORMULATIONS

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Article Received on
11 March 2019,

Revised on 01 April 2019,
Accepted on 22 April 2019,

DOI: 10.20959/wjpps20195-13780

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ABSTRACT

Antioxidants are compounds that have capability of either delay or inhibit the oxidation processes. It occur under the influence of oxygen or reactive oxygen species. Antioxidants are compound involved in the defense mechanism of the organism against the pathologies associated condition due to the attack of free radicals. There are several benefits of antioxidants like antioxidants may boost our brain function, antioxidants decreases oxidative stress, antioxidants prevent cancer, promote liver health, treat urinary tract infection, can treat acne, delay aging, can help bodybuilders. Antioxidant prevent the free radical reaction and protect the muscles from being damaged. Vitamin C have great role in tissue repair. The antioxidant activity of the drugs sample and the standard was measured on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picryl hydroxyl (DPPH) free radical activity method. The stable DPPH radical method is a widely used, relatively quick, most accepted and precise method for the evaluation of the free radical scavenging activity of the drug sample. All eight marketed hepatic formulation showed the capacity of scavenging of free radical. The lowest antioxidant activity is shown by Udiliv i.e. 8.97% and silybon shows the highest antioxidant activity i.e. 60.86%.

KEYWORDS: Antioxidant, Free radicals, oxidative stress, DPPH, Reactive oxygen species

1. INTRODUCTION

Antioxidants are compounds that have capability of either delay or inhibit the oxidation processes. It occur under the influence of oxygen or reactive oxygen species. Antioxidants

are compound involved in the defense mechanism of the organism against the pathologies associated condition due to the attack of free radicals.[1]

Mostly enzymes, like superoxide dismutase, catalase, and glutathione peroxidase shows the powerful antioxidant capacity which is also known endogenous antioxidant. The nonenzymatic antioxidant compound are uric acid, bilirubin, albumin, metallothioneins.[2] When endogenous antioxidant cannot have control over the oxygen free radicals, exogenous antioxidant is need as supplementary dietary, which contain an antioxidant compound as active principle. The most important exogenous antioxidants are vitamin E, vitamin C, β-carotene, vitamin E, flavonoids, mineral are well known. Vitamins, flavonoids, anthocyanin's, some mineral compounds are the natural sources from which exogenous antioxidant can derived.[3] There is growing interest in antioxidants area, which purpose to prevent the presumed deleterious effects of free radicals in the human body.[4]

Health benefit of antioxidant

Antioxidants are vitamins and minerals that protect our cell from being damaged by free radicals, it is molecules that attack or injured healthy cells, and make weaken to immune systems. Free radicals can leads to progressiveness of cancer, cardiovascular disease, brain dysfunction, cataracts, and some other related disease.[5] Therefore, antioxidants may help maintain the perfect health and wellbeing. Vitamins C and E, beta-carotene, flavonoids, lutein, catechins, which act as powerful antioxidants can be found in natural foods, as well as dietary supplements. Supplementary antioxidant should checked by doctors before used because sometimes it interact with other medication. While excessive in taking of vitamins and minerals sometimes it may be harmful.[6]

The mechanism of action of antioxidants

Initiation

LH + R· → L· + RH where LH represents the substrate molecule, for example, a lipid, with R· as the initiating oxidizing radical. Allyl radical L can rapidly react with oxygen to form a lipid peroxyl radical (LOO·).

Propagation

$L\cdot + O_2 \rightarrow LOO\cdot$

$LOO\cdot + LH \rightarrow L\cdot + LOOH$

The peroxyl radicals also further oxidize the lipid, the lipid hydroperoxides (LOOH), break down through a wide range of compounds[7], including alcohols, aldehydes, alkyl formates, ketones and hydrocarbons, and radicals, including the alkoxyl radical (LO·).

Branching

LOOH → LO· + HO·

2 LOOH → LOO · + LO· + H_2O

The breakdown of lipid hydroperoxides include transition metal ion, this reaction is alike to those hydrogen peroxide, which produce lipid peroxyl and lipid alkoxyl radicals.

Termination

Termination reactions include the combination of free radicals to form non radical products:

LO · + LO ·

LOO · + LOO ·

LO · + LOO ·

The Primary antioxidants, if present in small amounts, it delay or inhibit the initiation step. Peroxyl or alkoxyl radicals react with a lipid radical or inhibit the propagation step.[8]

L · + AH → LH + A·

LOO· + AH → LOOH + A·

LO· + AH → LOH + A·

Secondary or preventative antioxidants delay the rate of oxidative reaction process. This is attained by removal of substrate or singlet oxygen quenching.[9]

Methods of Total Antioxidant Capacity Assessment

The various analytical methods of evaluation of the antioxidant capacity are as follows.

Spectrometric Techniques

The DPPH Method

The DPPH radical is one of the few stable organic nitrogen radicals, which bears a deep purple colour. It is commercially available and does not have to be generated before assay like ABTS.

The principal of DPPH is based on measurement of reducing capacity of antioxidant towards DPPH. The capacity antioxidant is evaluated by electron spin resonance (EPR) or by

measuring the decrease of absorbance. Brand-Williams and co-workers reported the first use of decolouration method.[10] Antioxidant assays determine the loss of DPPH colour at 517 nm, interacting with test compounds.[11] And the reaction is monitored by a spectrometer.

Advantages/Disadvantages of the DPPH Assay

The test is simple and rapid and needs only a UV-vis spectrophotometer to perform, which probably explains its widespread use in antioxidant screening. However, interpretation is complicated when the test compounds have spectra that overlap DPPH at 515 nm. Carotenoids, in particular, interfere.[12] Use of DPPH to measure AOC is plagued by many drawbacks. The assay is not a competitive reaction because DPPH is both radical probe and oxidant. DPPH colour can be lost via either radical reaction or reduction as well as unrelated reactions, and steric accessibility is a major determinant of the reaction. Thus, small molecules that have better access to the radical site have higher apparent AOC with this test. DPPH is a stable nitrogen radical that bears no similarity to the highly reactive and transient peroxyl radicals involved in lipid peroxidation.[13] Many antioxidants that react quickly with peroxyl radicals may react slowly or may even be inert to DPPH due to steric inaccessibility. DPPH also is decolorized by reducing agents as well as H transfer, which also contributes to inaccurate interpretations of AOC. Thus, AOC is not fairly rated by the ability of antioxidants to react with DPPH.

2. MATERIAL AND METHOD

Table 1: Marketed Allopathic Hepatic Formulation, their active pharmaceutical ingredient (API) and manufactured company.

S.NO	DRUG	API	MANUFACTURED BY
1	HEPACURE	L-ornithine L-aspartate, silymarin, vit B complex, niacinamide, calcium	MISSION RESEARCH LABORATORIES PRIVATE LIMITED
2	HEPAFORD	L-ornithine L-aspartate, pancreatin	ALLKIND HEALTH CARE
3	HEPTIVA	L-ornithine L-aspartate, pancreatin	PRM LIFE SCIENCE PRIVATE LIMITED
4	MICROLIV FORTE	Metadoxine, silymarin, L-ornithine L-aspartate	MIC-MICRO LABS PRIVATE LIMITED
5	SOLYBONE-140	Silymarin	MICRO LABS LIMITED
6	UDCA 300	ursodeoxycholic acid	LOGOS PHARMA
7	UDILIV 300	ursodeoxycholic acid	ABBOT INDIA LIMITED
8	URSOFORD	ursodeoxycholic acid	ALLKIND HEALTH CARE

The allopathic hepatic formulations were purchased from local market of faridkot, Punjab.

Method for evaluating antioxidant activities.

Antioxidant Activity by DPPH Method

Accurately weighed 2mg of DPPH and rest all sample (Hepacure, Hpaford, Heptiva, Microliv Forte, Silybon, Udiliv, Ursoford, UDC 300) are weighed 10mg including ascorbic acid as standard and prepared in methanol. DPPH radical-scavenging activity was measured according to the method of Shimada *et al.* 1 ml of 0.1 mM freshly prepared DPPH solution in methanol was added to 1 ml of each sample, the mixture was shaken vigorously and left in the dark at room temperature for 30 min. The absorbance of the resultant solution was measured at 517 nm, using ascorbic acid as the standard. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation.

DPPH scavenging effect (% inhibition) = {(A0 –A1)/A0)*100} Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in presence of all of the extract samples.

3. RESULT AND DISCUSSION

Antioxidant is a molecule that inhibits the oxidation of other molecules. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidative reactions. There is growing interest in antioxidants capacity to prevent the human body from deleterious effects of free radicals.

Liver is the primary organ for the metabolism of any xenobiotic. During metabolism there is most possible chances to generate free oxidative radicals which causes oxidative stress to hepatic cell due to oxidative stress, hepatic cell may damage or death. Hence, the formulations prescribed for hepatic disorders should possess good antioxidant activity which easily terminate the chain reaction of free radical.

Table 2: Absorbance of standard, control and drug sample

Name	Sample	Absorbance
Standard	Ascorbic acid	0.011
Control	DPPH	0.925
Drug sample	Microlivforte	0.626
	Hepacure	0.641
	UDCA 300	0.815
	Ursoford	0.525
	Heptiva	0.795
	Udiliv	0.842
	Silybon 140	0.362
	Hepaford	0.739

Table 3: Antioxidant capacity of marketed hepatic formulation are summarized in table

S.No	Drug	Antioxidant capacity
1	Microlivforte	32.32%
2	Hepacure	30.70%
3	UDCA 300	11.89%
4	Ursoford	43.24%
5	Heptiva	14.05%
6	Udiliv	8.97%
7	Silybon 140	60.86%
8	Hepaford	20.11%

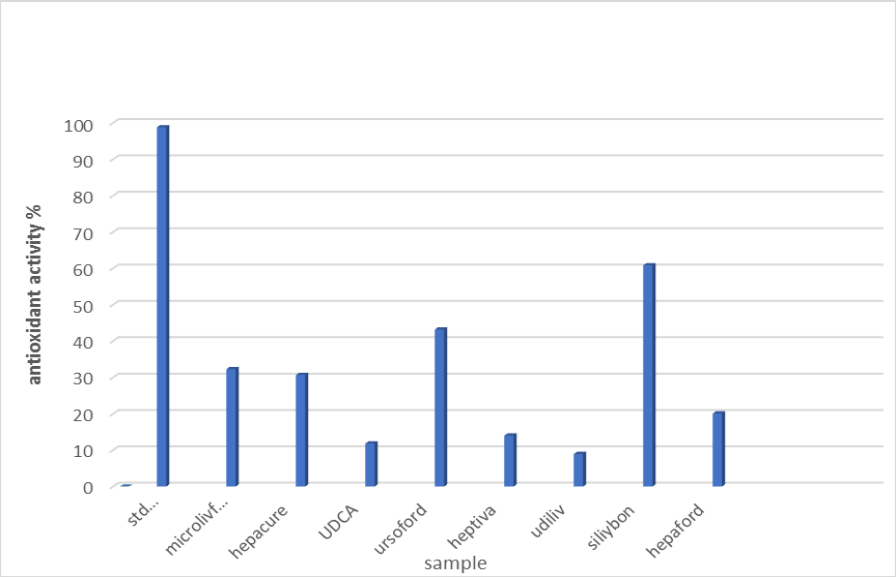

Figure 1: Antioxidant capacity percentage in chart.

The antioxidant activity of the drugs sample and the standard was measured on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picryl hydroxyl (DPPH) free radical activity method. The stable DPPH radical method is a widely used, relatively quick, most accepted and precise method for the evaluation of the free radical scavenging activity of the plant extract. 1, 1-diphenyl-2- picryl hydroxyl (DPPH) is a stable free radical and accepts an electron or hydrogen to become a stable molecule. Antioxidant on interaction with DPPH, transfer an electron or hydrogen atom to DPPH and thus neutralizing its free radical character. The degree of discoloration of DPPH indicates the scavenging activity of the plant extract. The determination of reduction capacity of DPPH radical is decreasing in its absorbance at 517 nm.

The reaction between antioxidant and free radical cause decrease in the absorbance of DPPH radical. Easily visualize change in colour from purple to yellow. The free radical.

DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). When an antioxidant reacts with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor and is reduced to the DPPHH and as consequence, the absorbance at 517 nm decreases from the DPPH to DPPH-H form; results in decolourization (yellow colour) with respect to the number of electrons captured. More the decolourization more is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of sample, hence DPPH is usually used as substance to evaluate the antioxidant activity.[14]

All at sample of marketed Hepatic formulations were assayed by DPPH radical scavenging method. The absorbance of each sample were taken at 517nm. Ascorbic acid had taken as standard sample and shows 0.011 absorbance at 517nm and at marketed hepatic formulation Microleforte, Hepacure, UDCA, Urosoford, Heptiva, Udiliv, Silybon, Hepaford shows 0.626, 0.641, 0.815, 0.525, 0.795, 0.842, 0.362, 0.739 respectively of absorbance.

According to absorbance, antioxidant activity were calculated of all each sample. In which standard sample (Ascorbic acid) shows 98.81% of antioxidant activity and marketed hepatic formulations Microlivforte, Hepacure, UDCA, Urosoford, Heptiva, Udiliv, Silybon, Hepaford shows 32.32%, 30.70%, 11.89%, 43.24%, 14.05%, 8.97%, 60.86%, 20.11% respectively of antioxidant activity. All taste sample marketed hepatic formulations shows satisfactory antioxidant activity.

In DDPH radical scavenging assay, antioxidant reacts with DDPH and converts from purple to yellow colour. The degree of discolouration indicates the radical scavenging potential of sample.[15]

4. CONCLUSION

Antioxidant activity of marketed hepatic formulation was evaluated by DDPH radical scavenging method. All eight marketed hepatic formulation showed the capacity of scavenging of free radical. The lowest antioxidant activity is shown by Udiliv i.e. 8.97% and silybon shows the highest antioxidant activity i.e. 60.86%. The reduction capability of DDPH radical is caused by the antioxidant because of the reaction between antioxidant, molecules and free radical, progress which result in the scavenging of the radical by hydrogen donation. This consist of preventing the occurrence of oxidative stress related diseases caused by the attack of free radical on key components like lipid and nucleic acid.

5. ACKNOWLEDGMENT

I am immensely thankful to University Centre of Excellence in Research, BFUHS, Faridkot (Punjab) for allowing us to do our project work and providing us with necessary support to complete this research work successfully.

6. REFERENCE

1. Carocho M, Ferreira IC. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food and chemical toxicology, 2013 Jan 1; 51: 15-25.
2. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition, 2002 Oct 1; 18(10): 872-9.
3. Litescu SC, Sandra AV, Eremia SAV, Diaconu M, and Tache A, Biosensors Applications on Assessment of Reactive Oxygen Species and Antioxidants. In Tech Rijeka Croatia, 2011.
4. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity, for estimating antioxidant activity, Songklanakarin Journal of Science Technology, 2004; 26: 211-219.
5. Poljsak B, Šuput D, Milisav I. Achieving the balance between ROS and antioxidants: when to use the synthetic antioxidants. Oxidative medicine and cellular longevity, 2013 Apr 29; 2013.
6. Park YK, Park E, Kim JS, Kang MH. Daily grape juice consumption reduces oxidative DNA damage and plasma free radical levels in healthy Koreans. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 2003 Aug 28; 529(1-2): 77-86.
7. Cheeseman, K. H., and Slater, T. F. An introduction to free radical biochemistry. British medical bulletin, 1993; 49(3): 481-493.
8. Madhavi, D. L., Deshpande, S. S., & Salunkle, D. K. Food antioxidants: technological, toxicological, and health perspectives. Food science and technology, 1996; 267-360.
9. Giardi, M. T., Rea, G., and Berra, B. Bio-farms for nutraceuticals. Functional food and safety control by biosensors. Preface. Advances in experimental medicine and biology, 2010; 698.
10. Brand-Williams. W., Cuvelier, M. E., and Berset, C. L. W. T. Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology, 1995; 28(1): 25-30.

11. Bondet V., Brand-Williams, W., and Berset, C. Kinetics and mechanisms of antioxidant activity using the DPPH. free radical method. LWT-Food Science and Technology, 1997; 30(6): 609-615.
12. Nomura, T., Kikuchi, M., Kubodera, A., and Kawakami, Y. Proton-donative antioxidant activity of fucoxanthin with 1,1-diphenyl-2-picrylhydrazyl (DPPH). IUBMB Life, 1997; 42(2): 361-370.
13. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of agricultural and food chemistry, 2005 May 18; 53(10): 4290-302.
14. Ilhami Gu lc¸ Zubeyr Huyut b, Mahfuz Elmastas c, Hassan Y and Aboul-Enein d, Radical scavenging and antioxidant activity of tannic acid, Arabian Journal of Chemistry, 2010; 3: 43–53.
15. Sreelatha S, Padma PR. Antioxidant activity and total phenolic content of Moringa oleifera leaves in two stages of maturity. Plant foods for human nutrition, 2009 Dec 1; 64(4): 303.