IN VIVO ANTIOXIDANT AND NEUROPROTECTIVE EFFECT OF *HIPPOPHAE RHAMNOIDES*. L.

SHIVAPRIYA SHIVAKUMAR[1], K. ILANGO[1], A. AGRAWAL[2], A. TRIGUNYAT[2], G.P.DUBEY[2]*

[1]Interdisciplinary School of Indian System of Medicine, SRM University, Kattankukathur, Tamilnadu, India, [2]Faculty of Ayurveda, Institute of Medical Science, Banaras Hindu University, Varanasi, UP, India. Email: spsk99@gmail.com, gpdubey13@gmail.com

ABSTRACT

Background: Oxidative stress leads to many disorders and this can be overcome using plants sources, rich in antioxidants that can reduce the damage caused by these stress markers. Prolonged oxidative stress to neurons in the brain leads to increased formation of lipofuscin. Lipofuscin is a yellow-brown product which accumulates in various cells like nerve cells, ganglia cells, heart and muscle cells and it is the end product of oxidation of lipids, protein and other biomolecules present in the cell and it is found to be a principal marker of brain vulnerability, stress, aging and related pathology.

Materials and Method: *Hippophae rhamnoides* is a rich source of anti-oxidants. Present study was conducted to evaluate the neuroprotective and antioxidant activities of *H. rhamnoides.* Sprague dawley rats of different age groups were used for the study. The neuroprotective activity of the plant was assessed by its ability to maintain the level of neurotransmitters. The antioxidant activity was measured from the level of stress markers and lipofuscin pigmentation. Lipofuscin pigment formation was estimated by a quantitative method based on fluorescence spectroscopy.

Results: It was observed that there was an increase in the level of the neurotransmitters in treated animals it is also seen the lipofuscin concentration was (6-8 times) less in *H. rhamnoides* treated animals when compared with normal aged animals.

Conclusion: The results conclude that *H. rhamnoides* has a therapeutic potential to act as a neuroprotective and antioxidant agent by reducing oxidative stress markers and lipofuscin pigments.

Keywords: Lipofuscin, *Hippophae rhamnoides*, Neuroprotective, Anti-oxidant, Oxidative stress markers, Aging.

INTRODUCTION

Brain is a metabolically active organ which utilizes nearly 20% of oxygen even at rest and has relatively reduced space for cellular regeneration, hence more susceptible to reactive oxygen species (ROS) damage. Studies have reported that oxidative damage is evident in age-related neurodegenerative diseases Alzheimer's disease (AD), Parkinson's disease (PD), and Amyotrophic Lateral Sclerosis (ALS), Huntington disease, Progressive Supranuclear Palsy, and Prion disorders have particularly been reported for ROS damage in the specific regions of the brain [1-3] Markers of lipid oxidation, malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) are found in the cortex and hippocampus in AD patients and in the substantial nigra of the PD cases [4-6]

Oxidative stress is responsible for wear and tear of the cells and excessive exposure of the cells to these free radicals can cause severe damage to it. It is evident from earlier studies that intrinsic oxidative stress and cell damage increases with age due to diminished antioxidant defenses or with an increase in mitochondrial dysfunction [7].A number of studies have shown an age-related increase in oxidative damage to molecules like lipid, protein and DNA which occurs in organisms ranging from invertebrates to humans [8-10].There is a wide variety of ROS found in the biological systems which are neutralized with the help of antioxidant enzymes. An increase in oxidative damage could result either from deficiencies in the normal cell antioxidant defenses or an increased production of free radicals.

In aged cells there is excessive oxidative stress and lack or deficiency of anti-oxidant enzyme, this leads to the excess lipofuscin deposition. These pigments are membrane-bound cellular waste, which can be neither degraded nor ejected from the cell but can only be diluted through cell division and subsequent growth. Postmitotic cells generally accumulate lipofuscin, which is an "aging pigment" has been considered as a reliable biomarker for the age of cells. Lipofuscin pigments are not uniformly distributed but are concentrated in specific regions in the aging brain [11].

Antioxidant like vitamins, flavonoids and polyphenolic compounds of plant origin has been extensively investigated as scavengers of free radicals and inhibitors of lipid peroxidation. *H. rhamnoides* is a natural reservoir of vitamins, minerals, antioxidants and flavonoids [12-14]. The *in-vitro* studies show that *H. rhamnoides* has strong antioxidant and immunomodulatory properties [15].The berries of the plant have carotenoids, Pro Vitamin A, tocopherols-Vitamin E, Vitamin K, Vitamin C, essential fatty acids, Vitamin B-12 and the leaves of *H. rhamnoides* contains flavonoids which show effective anti oxidant and anti inflammatory activity [16]. The leaves of *H. rhamnoides* were also considered for their antioxidant potential correlated to flavonoids and phenolic acids derivatives [17].

In the present study attempt has been made to find the efficacy of *H. rhamnoides* in alleviating the formation of lipofuscin by reducing the formation of ROS and RNS production in aged rats and also the effectiveness of *H. rhamnoides* to increase the level of neurotransmitters in the aged rat's brain.

MATERIALS AND METHODS

Chemicals

Thiobarbituric acid, 1,1,3,3-tetramethoxypropane, glutathione reduced and quercetin and other chemical required for the measurements of the neurotransmitters and ChAT was procured from Sigma Aldrich Ltd USA (Biotech India local distributors). The entire chemicals used were of analytical grade.

Animals and Care

Adult male Sprague dawley rats of various age groups (4-10, 10-12, 20-22 and 22-24 month old) have been procured from central animal house of SRM University. The animals were housed in stainless cages with 4 rats kept per cage in limited-access facility. The animal house was maintained at 23ºC±2ºC with a relative humidity at 50%±10%. Artificial light in cage was used for 12 hours in each day. Sterilized drinking water and standard chow diet (ad libitum) were given to animals during the whole period of the experiment. The guide for the care and use of laboratory animals was adopted from the international guidelines to carry out the present animal study. Institutional animal ethical committee (IAEC), SRM University, Kattankulathur, Tamilnadu, provided ethical clearance for the experiment. The animals were acclimatized in laboratory condition for one week prior to the experiment.

Experimental design

The Sprauge Dawely rats are divided into 4 following groups, with 4 rats in each group.

Group-I, 4-6 months old rats were used, (normal control group),

Group-II, 10-12 months old rats were used (normal control for significant age increase comparison),

Group-III, 20-24 months old rats were used (disease control group),

Group-IV, 20-22 months old rats were used (treated group) given test drug *H. rhamnoides* (250mg/kg) for 6 weeks.

Test drug formulation

The dose dependent activity of the plant extract was done using different plant extract concentration 150, 250, 350mg/ml of which 250 mg/ml showed the maximum activity hence this was taken as the active dose concentration for the present study. The test formulation consists of hydro-alcoholic extracts of *H. rhamnoides* fruits 125mg/kg body weight and Leaves 125mg/kg body weight. Were orally administered (through gastric incubation) for 6 weeks. 0.5ml/kg is taken as constant dose volume.

Tests conducted

Tests were conducted in the experimental animals with standard protocol. The rats were killed by decapitation, tissue sample were taken from brain and analyzed for lipofuscin pigment and the lipofuscin concentration was calculated as relative fluorescence units per gram of wet tissue [18]. Acetylcholine and Nor-adrenaline estimation have been performed by fluorometric method [19]. Dopamine and Glutamate estimated by HPLC method. The choline acetyltransferase (ChAT) activity was measured by radiometric method [20]. Lipid peroxidase activity is being analyzed by TBARS method [21]. Total glutathione content was measured by colorimetric method [22] and Active avoidance test [23].

RESULTS

Statistical tools were used to compare the results of the experimental groups. The variability of result is expressed as the mean ± SEM. Statistical analysis for the mean difference between the experimental groups is done with by using Students "**t - test**".

Beneficial role of *H. rhamnoides* on neurotransmitters

Table 1, 2 illustrates the level of neurotransmitters (acetylcholine, glutamate, dopamine and nor-adrenaline) with age. The level of

Acetylcholine in group IV was 16.128±2.056 and group III was 12.985±1.853, there was an increase in the level but the *p* value between groups was less significant (*P*<0.05). The level of glutamate in group IV was 15.128±2.174 and group III was 20.42±4.110, there was a decrease in the level and when the *p* value was compared between groups it was less significant (*P*>0.05). The level of dopamine in group IV was 0.352±0.093 and group III was 0.268±0.045, there was an increase in the level but when the *p* value was compared between the groups it was significant (*P*<0.01). The level of nor-adrenalin in group IV was 0.319±0.085 and group III was 0.243±0.044, there was an increase in the level, when the *p* value was compared between group III and IV it was less significant (*P*>0.05). From the result it is seen that the drug increases the level of neurotransmitters in treated group but the increase was less significant.

Effect o*f H. rhamnoides on* retention of active avoidance learning and ChAT activity

Table 2 explains the age consistent ChAT enzyme activity and retention of active avoidance learning (RAAL) score. The ChAT enzyme activity and RAAL was higher in the *H. rhamnoides* treated group. The level of ChAT enzyme activity in group IV was 19.885±3.012 and group III was 15.316±2.112, there was an increase in the level, when the *p* value was compared between group III and IV and it was less significant (*P*>0.05). The retention of active avoidance score in group IV was 11.780±2.851 and group III was 14.251±2.752, there was an decrease in the level and when the *p* value was compared between group III and IV and it was less significant (*P*>0.05). Hence the test drug acts efficiently on the cognitive process and increases the learning capacity of animals in the treated group.

Age consistent status of oxidative stress markers and role of *H. rhamnoides*

The status of stress markers with age is shown in table 3. The level of oxidative stress markers was measured by TBARS assay the level of stress marker in group IV was 67.913±13.805 and group III was 88.942±9.346, there was a considerable decrease in the level, while comparing the *p* value between group III and IV and it was less significant (*P*>0.05). The level of glutathione enzyme in group IV was 48.993±9.226 and group III was 32.758±5.902, there was an increase in the level of glutathione, but while comparing the *p* value between group III and IV, it was less significant (*P*>0.05). Thus the test drug *H. rhamnoides* acts as an active antioxidant agent by reducing the level of TBARS and increasing the level of the anti oxidant enzyme Glutathione in the treated group.

Table 1: Age consistent status of neurotransmitter and beneficial role of *H. rhamnoides*

Groups	Acetylcholine (nm/gm)	Glutamate (µmol/gm wt. tissue)	Dopamine (µg/gm)
Group-I (4-6 month old rats)*	21.342±3.086	10.94±2.153	0.434±0.072
Group-II (10-12 month old rats)**	17.934±2.705	16.83±3.412	0.330±0.028
Group-III (20-24 month old rats)***	12.985±1.853	20.42±4.110	0.286±0.045
Group-IV (20-22 month old rats) + *H.rhamnoides* treated ****	16.128±2.056	15.128±2.714	0.352±0.093

Comparison:

*vs**	P>0.05	P>0.01	P<0.01
vs*	P<0.001	P>0.05	P<0.01
vs*	P<0.05	P>0.05	P<0.01

Table 2: Age consistent neurotransmitter activity including behavioral pattern and effect of *H. rhamnoides*

Groups	Nor-adrenaline (µg/gm)	ChAT activity (nmol/mg protein/hour)	RAAL (score)
Group-I (4-6 month old rats)*	0.458±0.062	21.842±3.014	7.910±1.336
Group-II (10-12 month old rats) **	0.281±0.036	17.442±2.498	10.886±2.119
Group-III (20-24 month old rats) ***	0.243±0.044	15.316±2.112	14.251±2.752
Group-IV (20-22 month old rats) + *H.rhamnoides* (N=4)****	0.319±0.085	19.885±3.012	11.780±2.851

Comparison:

*vs**	P<0.01	P>0.05	P>0.05
vs*	P>0.05	P>0.05	P>0.05
vs*	P>0.05	P>0.05	P>0.05

Table 3: Age consistent status of oxidative stress markers and effect of *H. rhamnoides*.

Groups	TBARS (n.mTBARS/g.tissue)	Glutathione (mg GSH/mg. protein)
Group-I (4-6 month old rats)*	36.852 ±6.014	65.163±8.891
Group-II (10-12 month old rats)**	63.942±7.335	43.992±10.801
Group-III (20-24 month old rats)***	88.942±9.346	32.758±5.902
Group-IV (20-22 month old rats) + *H.rhamnoides* ****	67.913±13.805	48.993±9.226

Comparison:

*vs**   P<0.01   P<0.05

vs*   P>0.05   P>0.05

vs* P>0.05   P>0.05

Table 4: Age consistent lipofuscin pigments and beneficial role of *H. rhamnoides* extract

Groups	Lipofuscin (Relative Fluorescence/g tissue ±SEM)
Group-I (4-6 month old rats)*	429.886±72.539
Group-II (10-12 month old rats) **	768.364±116.993
Group-III (20-24 month old rats) N=4 ***	1129.649±274.923
Group-IV (20-22 month old rats) + *H.rhamnoides* (N=4)****	816.345 ±146.760

Comparison:

*vs**   P>0.05

vs*   P>0.05

vs* P>0.05

Age consistent lipofuscin pigmentation and beneficial role of *H. rhamnoides*

Table 4 shows the amount of lipofuscin pigment formed in relation to age. Oxidative stress increases with age due to decrease in the level of biological antioxidants leading to large amount of lipofuscin deposition. The concentration of lipofuscin pigments in group IV was 816.345±146.760 and group III was 1129.649±923, there was a considerable decrease in the level of lipofuscin, while comparing the *p* value between group III and IV and it was less significant (*P*>0.05). Thus *H. rhamnoides* is a natural antioxidant prevents accumulation of lipofuscin pigments in aged cells by reducing the oxidative stress.

DISCUSSION

Studies have revealed that oxidative stress is associated with the pathogenesis of a number of diseases mainly neurodegenerative disorders, ischemia, etc. Oxidation of biomolecules due to ROS activity may lead to cellular dysfunction which has been noticed in studies [24-27]. A balance between the pro-oxidant and anti-oxidant levels is required for normal physiological conditioning of the cells, and its imbalance may lead to increased production of ROS and RNS. In turn, these ROS and RNS react with biomolecules like proteins, lipids, carbohydrates, DNA and RNA [28]leading to oxidative damage of the cells by producing large amount of lipofuscin deposition. Our study has also revealed that the ROS increases with age and causes excess oxidation of biomolecules which, can be seen from the increased level of lipofuscin Pigment in the group III.

There are evidences that natural anti-oxidants, such as carotenoids, tocopherols and flavanoids, can effectively prevent and cure oxidative stress related disease [29]. Flavonoids show effective anti-oxidant property which protects against disease and aging process [30]. Studies have shown that the antioxidant activity of *H. rhamnoides* is due to vitamins, trace elements, amino acids and other bioactive substances, such as β-carotene, zeaxanthin, lycopene, flavonoids, folic acid, , fatty acids, tannic acid polyphenols present in it and also the fruits of *H. rhamnoides* have antioxidant properties and they can protect human body against the damaging effect of oxidized radicals [31].

Glutathione plays a key role in maintaining the redox homeostasis in the cell [32] and the age associated glutathione depletion leads to shifting of redox status towards the more oxidized state which alters the receptors, proteins kinase, transcription factors and several signaling cascade. The magnitude of an oxidative event is associated with cellular proliferation, differentiation, and death which in turn depend on the GSH content of the cell [33]. The results of the study shows increased TBARS content and decreased glutathione content in aged or disease control brain. The drug reverse the glutathione content to significant level thus helps in maintaining the redox status to normal.

Antioxidants are bioactive substances that prevent oxidation or inhibit reactions promoted by triterpene oxygen or peroxides and thereby protect cells from damage caused by oxidative stress. Free radicals like ROS, hydroxyl radicals cause damage and are also responsible for the degeneration of the nerve cells causing aging [34]. ROS can easily initiate the lipid peroxidation of the membrane lipids which causes damage of the cell membrane of phospholipids, lipoprotein by propagating a chain reaction cycle [35-36].

The neuroprotective effect of the drug attributed anti-oxidant activity which is influenced through glutathione peroxidase (GSH) a key anti-oxidant enzyme. Our observations revealed that supplementations with plant extracts significantly influenced the concentration LFP during the therapy period. In brief, LFP, as the end product of oxidative damage, exhibit marked changes when determined in relation to ageing process. The *H. rhamnoides* extract with its micronutrient properties have shown significant effect on the anti-oxidant defense mechanism in aged brain. Further the test drug show maximum bioactivity through anti-oxidant defense mechanism in aged brain by reducing the lipid peroxidation and increasing glutathione content required for free radical scavenging activity.

Several studies have been investigated on the hypothesis behind the decline of the neurotransmitter on aging and the mechanism underlying in cognitive, behavioral, and motor changes during normal aging and related complications [37]. Studies support significant relationship between ageing, cholinergic impairment, and cognitive decline. In the present study the level of acetylcholine and ChAT activity is found to decrease in the aged brain also support significant relationship between ageing, cholinergic impairment and cognitive decline. The test drug increases the acetylcholine and ChAT activity by stimulating the acetylcholine synthesis.

Apart from cholinergic disruption abundant evidences revealed significant monoaminergic deficits with progressive ageing [38]. Studies have shown that metabolism of monoamine neurotransmitter changes significantly when ageing, such as reduction of nor-epinephrine (NE) and dopamine (DA) in the brain stem of aged rat's, etc., which may be an important cause of ageing [39]. In the present study also there is a reduced content of NE, DA in the cortex of the aged rats. The extracts of *Hippophae rhamnoides* increases the level of these neurotransmitters, thus the result implies that the test drug may significantly alleviate age related neurodegenerative complications by modulating the neurotransmitter system

It is seen that natural products have proved to be a great source of biologically active compounds [40]. Recently researches on medicinal plants have attracted a lot of attention and there is large evidence to demonstrate the potential of medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases [41]. In the present study, it is evident that the plant *H. rhamnoides* which is rich in flavonoids, tocopherols, carotenoids and vitamins proved to be a neuroprotective and natural antioxidant and can effectively reduce the formation of ROS and RNS which in turn can help in alleviating the formation of lipofuscin in aged rat's brain.

CONCLUSION

The test drug show protective effect on aging and age related pathologies by ameliorating various pathways such as neurochemical modulation and oxidative stress. In the present study a positive correlation has been established between TBARS and GSH content with the neurotransmitter system and cognitive decline in aged rat's brain (cortex), and the treatment with *H. rhamnoides* extract significantly prevented this redox imbalance. The study concludes that *H. rhamnoides* is an antioxidant *a*gent, very effective in controlling the ROS and RNS production, and thus alleviates the formation of lipofuscin in the brain cortex region. Thus the test drug is a neuroprotective agent has it increases and maintains the level of neurotransmitter and prolongs aging process.

ACKNOWLEDGEMENT

REFERENCES

1. Albers DS, Augood SJ. New insights into progressive Supranuclear palsy. Trends in Neurosciences. 2001; 24: 347-353.
2. Butterfield DA, Kanski, J. Brain protein oxidation in age-related neurodegenerative disorders that are associated with aggregated proteins. Mechanism of Ageing and Development. 2001; 122: 945-962.
3. Kim JI et al., Oxidative stress and neurodegeneration in prion diseases. Annals of the New York Academy of Sciences. 2001; 928: 182-186.
4. Hensley K, Hall N, Subramaniam R, Cole P, Harris M, Aksenov M, Aksenova M, Gabbita SP, Wu JF, Carney JM, Lovell M, Markesbery WR and Butterfield DA. Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. Journal of Neurochemistry. 1995; 65: 2146–2156.
5. Butterfield DA, Castegna A, Lauderback CM, and Drake J. Evidence that amyloid beta-peptide-induced lipid peroxidation and its sequelae in Alzheimer's disease brain contribute to neuronal death. Neurobiology of Aging. 2002; 23: 655–664
6. Good PF, Werner P, Hsu A, Olanow CW, and Perl DP. Evidence for neuronal oxidative damage in Alzheimer's disease. American Journal of Pathology. 1996; 149: 21–28.
7. Cassarino DS, Bennett (Jr), JP. An evaluation of the role of mitochondria in neurodegenerative diseases: mitochondrial mutations and oxidative pathology, protective nuclear responses, and cell death in neurodegeneration. Brain Research Review. , 1999; 29: 1-25.
8. Warner HR, Superoxide dismutase, aging and degenerative disease. Free Radical Biology of Medicine, 1994; 17: 249–258.
9. Bohr VA, Anson RM. DNA damage, mutation and fine structure DNA repair in aging. Mutation Research. 1995; 338: 25–34.
10. Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. Science. 1996b; 273: 59–63.
11. Dan Riga, Sorin Riga, Florin Halalau, and Francisc Schneider. Brain Lipopigment Accumulation in Normal and Pathological Aging; Annals of the New York Academy of Sciences. 2006; 1067: 158-163.
12. Deepak D, Maikhuri RK, Rao KS, Lalit K, Purohit VK, Manju S. Basic nutritional attributes of *Hippophae rhamnoides* (sea buckthorn) populations from Uttarakhand Himalaya. India Current Science. 2007; 92: 1148–1152.
13. Hibasami H, Mitani A, Katsuzaki H, Imai K, Yoshioka K, & Komiya T. Isolation of five types of flavonol from *H.rhamnoides* and induction of apoptosis by some of the flavonols in human promyelotic leukemia HL- 60 cells. International Journal of Molecular Medicine. 2005; 15: 805–809.
14. Shah AH, Ahmed D, Sabir M, Arif S, Khaliq & Batool F. Biochemical and nutritional evaluations of *H.rhamnoides* (*Hippophae rhamnoides* L. spp. Turkestanica) from different location of Pakistan. Pakistani Journal of Botany. 2007; 39: 2059–2065.
15. Geetha S, Ram MS, Singh V, Ilavazhagan G, & Sawhney RC. Effect of *Hippophae rhamnoides* on sodium nitroprusside induced cytotoxicity in murine macrophages. Biomedicine and Pharmacotherapy. (2002a); 56: 463–467.
16. Geetha S, Sai RM, Singh V, Ilavazhagan G, & Sawhney RC. Antioxidant and immunomodulatory properties of *H.rhamnoides* (*Hippophae rhamnoides*)—An in vitro study. Journal of Ethnopharmacology. (2002b); 79: 373–378.
17. Upadhyay NK, Kumar R, Siddiqui MS, Gupta A. Mechanism of wound healing activity of Hippophae rhamnoides L. leaf extract in experimental burns. Evidence based Complementary and Alternative Medicine. 2011; doi:10.1093/ecam/nep 189.
18. Csallany AS & Ayaz KL. Quantitative determination of organic solvent soluble lipofuscin pigments in animal tissues. Lipids. 1976; 11: 412-417.
19. Speeg KV. A sensitive and specific fluorometric method for the determination of acetylcholine. In: Choline and Acetylcholine: Handbook of Chemical Assay Methods. Edited by Hanin I, Raven Press, New York, pp, 1974; 129-133.
20. Chang, C. A change in the sub-cellular distribution of nor adrenaline in the rat isolated vas deferens affected by nerve stimulation. International Journal of Neuropharmacology. 1964; 3: 643-649.
21. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Annals of Biochemistry. 1979; 95: 351-58.
22. Ellman Gl. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics. 1959 May; 82 (1):70–77.
23. Sen AP, and Bhattacharya SK. Effect of selective muscarinic receptor agonists and antagonists on active-avoidance learning acquisition in rats. Indian Journal of Experimental Biology. 1991; 29: 136-139,
24. Aksenova M, ButterWeld DA, Zhang SX, Underwood M, Geddes JW. Increased protein oxidation and decreased creatine kinase BB expression and activity after spinal cord contusion injury. Journal of Neurotrauma. 2002; 19: 491–502.
25. Mark RJ, Lovell MA, Markesbery WR, Uchida K, Mattson MP. A role for 4-hydroxynonenal, an aldehydic product of lipid peroxidation, in disruption of ion homeostasis and neuronal death induced by amyloid beta-peptide. Journal of Neurochemistry. 1997; 68: 255–264.
26. Markesbery WR. Oxidative stress hypothesis in Alzheimer's disease. Free Radical Biology of Medicine. 1997; 23: 134–147.
27. Smith MA, Richey PL, Taneda S, Kutty K, Sayre LM, Monnier VM, Perry G. Advanced Maillard reaction end products, free radicals, and protein oxidation in Alzheimer's disease. Annals of the New York Academy of Sciences. 1994; 738: 447–454.
28. Halliwell B. Oxidative stress and neurodegeneration: where are we now? Journal of Neurochemistry. 2006; 97: 1634–1658.
29. Vitagligone P, Morisco F, Caporasco and Fogliana V. Dietary antioxidant compounds and liver health. Critical reviews in food science and nutrition. 2004; 44: 575-586.
30. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000; 408: 239-247.
31. Yang B and Kallio H. Composition and physiological effects of *H. rhamnoides* lipids. Trends in food science and technology, 2002; 13: 160-167.
32. Shukitt–Hale B, Youdin KA, Joseph JA. Critical reviews of oxidative stress and aging: advances in basic sciences, diagnostics and intervention, world scientific publishing, Singapore 2003.

33. Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radical Biology of Medicine. 2001; 30: 1191-1212.
34. Halliwelli B. Antioxidants and human disease: A general introduction. Nutritional reviews. 1997; 55: 44-52.
35. Maxwell SR. Prospects for the use of antioxidant therapies. Drugs.1995; 49: 345-361.
36. Braca A, Sortino C, Politi M, Morelli I, Mendez J. Antioxidant activity of flavonoids from *Licania licaniaeflora.* Journal of Ethnopharmacology. 2002; 79:379-381.
37. Buccafusco AD, Hall DA. Tissue vitamin E and lipofuscin accumulation with age in the mouse. Journal of Gerontology. 1981; 36:529-533.
38. Mazer C, Muneyyirei J, Taheny K. Serotonin depletion during Synaptogenesis lead to decreased synaptic density and learning deficits in the adult mice: a possible model of neurodevelopment disorders with cognitive deficits. Brain research.1997; 760: 68-73.
39. Lee JI, Chang CK, Liu IM, Chi TC, yu HJ, Cheng JT. Changes in the endogenous monoamines in the aged mice. Clinical and Experimental Pharmacology and Physiology. 2001; 28: 285-289.
40. D. Radhika, C. Veerabahu, R. Priya. *In vitro* Studies on Antioxidant and Haemagglutination Activity of Some Selected Seaweeds International Journal of Pharmacy and Pharmaceutical Sciences. 2013; Vol 5, Issue 1.
41. P. Neelavathi, P. Venkatalakshmi And P. Brindha Antibacterial Activities Of Aqueous And Ethanolic Extracts Of Terminalia Catappa Leaves And Bark Against Some Pathogenic Bacteria. International Journal Of Pharmacy And Pharmaceutical Sciences. 2013; Vol 5, Issue 1.