Int J Pharm Bio Sci 2014 JAN; 5(1): (P) 731 - 738

Research Article **Medicinal Chemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EFFECT OF *Artemisia vulgaris* LEAF EXTRACT ON ANTIOXIDANT STATUS OF PRIMARY CHICK EMBRYO FIBROBLASTS

SHARMILA, K. AND PADMA, P.R.*

Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore – 641 043

ABSTRACT

An imbalance between the pro-oxidants and/or free radicals on one side and antioxidants on the other results in oxidative stress that has shown to play a role in the etiology and pathophysiology of various human diseases. Antioxidant defense systems, both enzymic and non-enzymic scavenge the free radicals formed continuously in the body. Natural products of plant origin are gaining much importance in recent years for their pharmacological properties including antioxidant and antitumor activity. The present study focuses on the use of the methanolic extract of *Artemisia vulgaris* leaf extract on the enzymic and non-enzymic antioxidant status of chick embryo fibroblasts subjected to oxidative stress. The leaf extract of *Artemisia vulgaris* possess certain active ingredients that improve the antioxidant status in primary cells subjected to oxidative stress. Therefore, this experimental evidence suggests the use of the candidate plant for the treatment of human pathologies in which free radicals play a major role.

KEYWORDS: ***Artemisia vulgaris*, enzymic antioxidants, non-enzymic antioxidants, primary chick embryo fibroblasts**

PADMA, P.R.
Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore – 641 043

INTRODUCTION

When the scavenging capacity of the antioxidants exceeds the generation of reactive oxygen and other radical species, it results in oxidative stress [1]. Free radicals are molecules with one or more unpaired electron in their outermost orbit and include hydroxyl radical, nitric oxide and superoxide. Free radical toxicity has been implicated in the biomolecular damage of the proteins, lipids and DNA [2]. Increase in reactive oxygen species (ROS) has been linked to aging process and also to the etiopathogenesis of aging-related diseases, such as cancer, diabetes, atherosclerosis and degenerative diseases. Excess ROS has been deleterious to normal cells [3]. Various natural antioxidants are present within the body to scavenge the free radicals and prevent from oxidative damage to the structural integrity of the biomolecules. Antioxidant defense system involves both enzymatic intracellular enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase as well as non-enzymatic strategies that are mostly derived from dietary sources like Vitamin A, Vitamin C, Vitamin E and carotenoids [4]. The human body produces antioxidants naturally, but do not show 100% effective scavenging. Thus, the intake of foods and herbs rich in antioxidants is also essential for health benefits. Fruits, vegetables and medicinal herbs are the richest sources of antioxidant compounds [5].

Artemisia vulgaris belongs to the Asteraceae family having traditional use as herbal medicine for treating problem related to stomach and menstrual problems. It is also used in the Indian system of medicine as an emmenagogue, antihelmintic, antiseptic, antispasmodic and in the treatment of respiratory and nervous diseases [6]. There is less scientific validation of the plant for its antioxidant activity. Previous study has shown the methanolic extract of *A. vulgaris* leaves to be potent scavenger of free radicals when compared to the aqueous and chloroform extracts [7]. 0.1mg of the methanolic extract was used for the study based on the dose optimized in HepG2 cells [8]. Chick embryo fibroblasts represent a model of non-transformed cells and also a cost effective model to study the effect of the plant extract on primary culture. Several studies have used primary cultures as the test system for studying the medicinal property of plant extracts [9]. Hence, this study focuses the antioxidant potential of methanolic extract of *Artemisia vulgaris* leaves on primary chick embryo fibroblast.

MATERIALS AND METHODS

Plant materials and extraction

The plant sample was collected from Coimbatore and was grown within the university campus as pot culture. The plant was identified and certified by the Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore. Fresh leaves were collected, cleaned and extracted using methanol (1:10, w/v). The extract was evaporated to dryness and dissolved in minimum quantity of dimethyl sulfoxide.

Culturing of chick embryo fibroblast

The live embryo was seen as a shadow when holding a fertilized egg in the bright light source. An egg containing a live 8^{th} or 9^{th} day-old chick embryo was taken in a beaker with the blunt end up and swabbed with 70% alcohol. The blunt end of the egg was carefully punctured with the point of a sterile scissors and a circle of shell was cut away carefully to expose the underlying membrane called chorioallantois. The chorioallantoic membrane was carefully cut with a second pair of sterile scissors and removed to expose the embryo. The embryo was gently lifted by the neck using a sterile hook or a bent glass rod, and placed in a 100mm petridish containing PBS. It was then washed several times with PBS by transferring the embryo to fresh petriplates. After removal of all yolk and/or blood, the embryo was transferred to a clean dish with PBS. The head, limbs and viscera were removed using two sterile forceps. Care was taken to remove the entire limb by pulling at the proximal end. The remaining tissues of the embryo were taken to yet another sterile dish and washed with PBS. Then the embryo was finely minced with scissors and transferred to a flask containing PBS. After allowing the tissue pieces to settle, the PBS was removed with a sterile pipette. 2ml

of trypsin-EDTA (PAA, Austria) was added and the suspension was stirred gently for 15 to 20 minutes. The pellet was resuspended in fresh DMEM (PAA, Austria) with 10% FBS (PAA, Austria). From this, 20µl of the culture was taken to determine cell count and viability by trypan blue exclusion in a haemocytometer. Then the cells were seeded in 25cm^2 sterile tissue culture flask containing complete medium to a final concentration of 10^5 live cells/ml. The tissue culture flasks were incubated in a CO_2 incubator (Napko, UK) in a 5% CO_2 and 95% humidity atmosphere. After the cells had attained confluent growth, the cells were trypsinized using trypsin-EDTA and 10^3 cells were used for each assay.

Treatment groups

The treatment group set up were negative control without plant extract (0.1mg) and oxidant H_2O_2 (200µM) and a positive control group with primary chick embryo fibroblasts and oxidant. Test groups were set with the presence of the plant extract in the absence/presence of the oxidant. All the respective groups were incubated for 1 hour at 37°C.

Enzymic and non-enzymic antioxidants assays

The enzymic parameters, superoxide dismutase (SOD) [10], catalase (CAT) [11], peroxidase (POD) [12] and glutathione S-transferase (GST) [13] and the non-enzymic parameters, levels of vitamin C [14], vitamin E [15], vitamin A[16] and reduced glutathione [17] was determined as described earlier with slight modifications. In brief, 1 X 10^3 cells were taken for each treatment groups. The cells after 1 hr exposure to the oxidant were homogenised and 20µl of the sample was taken for each assay. The assay protocol was scaled down to µl volume of the reagents.

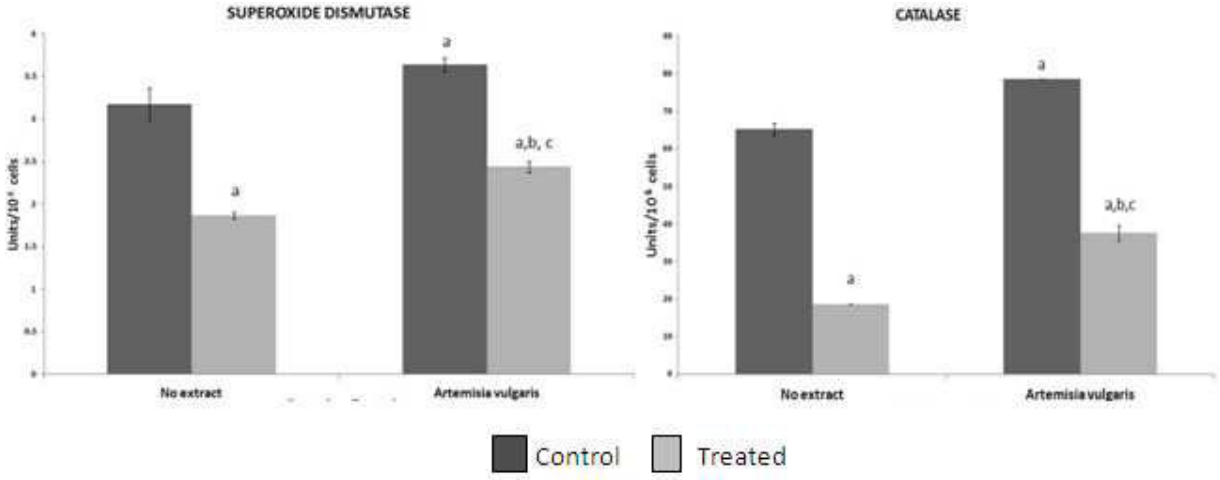
RESULTS AND DISCUSSION

Enzymic antioxidants

Superoxide dismutase (SOD), glutathione peroxidase, and catalase (CAT) are the preventive antioxidants and the first line of defense against ROSs. SOD, a key enzyme in the prevention of oxidative stress, catalyzes the removal of superoxide radical anion ($O_2^{-\bullet}$) to hydrogen peroxide (H_2O_2) that is further reduced by the catalase enzyme to water molecule [18]. Catalase, a tetrameric enzyme plays a major role in antioxidant defense by converting hydrogen peroxide to form water and molecular oxygen in the peroxisomes [19]. The biological function of peroxidase is to remove hydrogen peroxides and other hydroperoxides produced to protect the body against lipid peroxidation and DNA damage [20]. Glutathione S-transferase is multifunctional isoenzyme that can detoxify xenobiotics and endogenous metabolites primarily by catalyzing their conjugation with GSH [21]. The activities of the major enzymic antioxidants were assayed in the homogenate of the chick embryo fibroblasts after exposure to H_2O_2 in the presence/absence of the leaf extract. The enzymes assayed were SOD, CAT, POD and GST (Figure 1 and 2). Reduction in the enzymic activities was seen in the presence of the oxidant. Significant increase ($P<0.001$) in the activities of all the enzymes was seen when treated with the methanolic extract of *A. vulgaris* leaves. A slight elevation was also seen in the oxidant treated groups in the presence of the leaf extract when compared to the untreated group. The levels of different enzymatic and non-enzymatic antioxidants in the rat tissues increased significantly after treatment with the methanolic extract of *Alternanthera brasiliana* (L.) Kuntze leaves [22]. Among various extracts, methanolic extract of *Zea mays* leaves showed an improvement in the antioxidant status in the goat liver slices when exposed to oxidative stress *in vitro* [23]. Similarly, methanolic leaf extract of *Sphenocentrum jollyanum* caused a significant increase in the activities of serum and liver catalase, SOD and glutathione in *P. berghei* infected mice suggesting the antioxidant activity of the leaf extract [24]. Treatment with the methanolic extract of *Castanopsis indica* in a dose dependent manner significantly increased the CAT and SOD levels in the liver and kidney tissue as compared with Ehrlich ascites carcinoma control animals [25]. Also, a significant increase in the catalase activity was seen in the presence of the ethanolic extract of *Cocculus hirsutus* in ethanol induced ulcer rats [26].

Figure 1

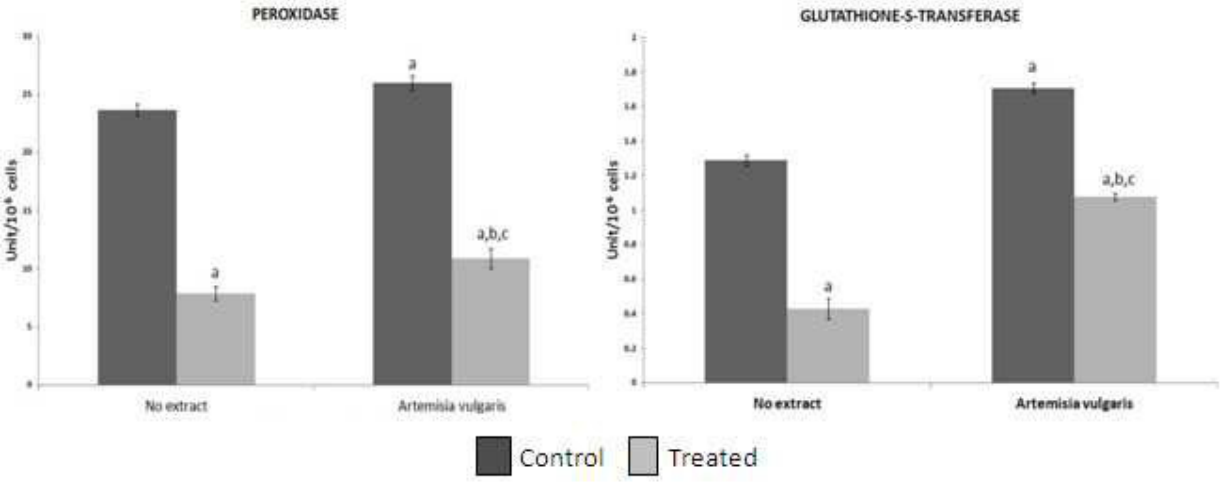
Effect of Artemisia vulgaris leaf extract on superoxide dismutase and catalase activity in primary chick embryo fibroblasts exposed to H_2O_2 in vitro



The values are mean ± S.D of triplicates
1 Unit = Amount of enzyme that causes 50% reduction in NBT oxidation for SOD
1 Unit = Amount of enzyme required to decrease the absorbance at 240nm by 0.05 units for catalase
a – Statistically significant (P<0.001) compared to untreated control
b – Statistically significant (P<0.001) compared to H_2O_2 alone treated group
c – Statistically significant (P<0.001) compared to the respective plant extract treated group

Figure 2

Effect of Artemisia vulgaris leaf extract on peroxidase and glutathione S-transferase activity in primary chick embryo fibroblasts exposed to H_2O_2 in vitro



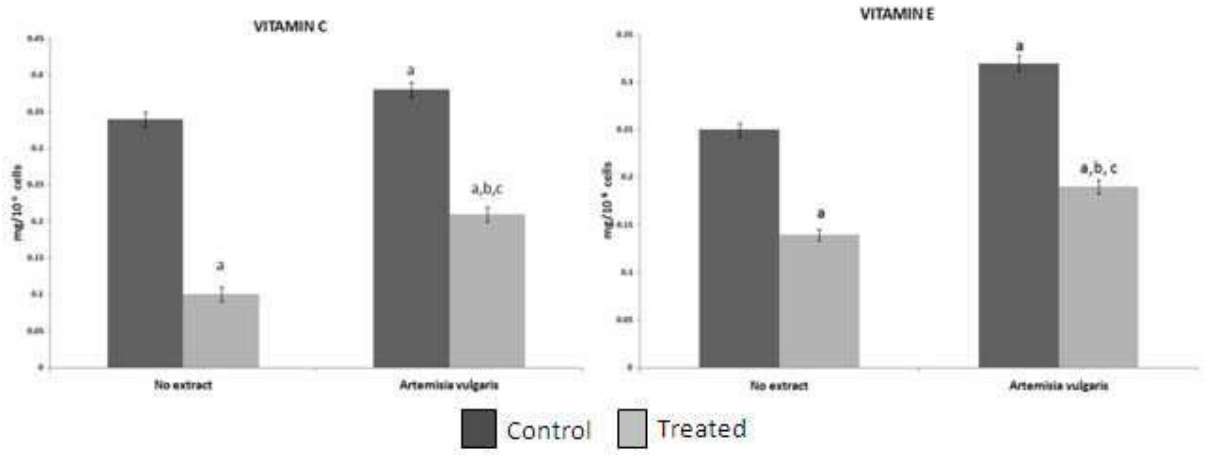
The values are mean ± S.D of triplicates
1 Unit = Change in absorbance at 430nm per minute for peroxidase
1 Unit = moles of CBNB conjugate per minute for GST
a – Statistically significant (P<0.001) compared to untreated control
b – Statistically significant (P<0.001) compared to H_2O_2 alone treated group
c – Statistically significant (P<0.001) compared to the respective plant extract treated group

Non-enzymic antioxidants

The second line of defense against free radicals damage is the non-enzymic antioxidant systems. Vitamin C is a water-soluble antioxidant present in the form of ascorbic anion in the system. It acts a scavenger of free radicals and helps in the regeneration of α-tocopherol [27]. Vitamin E is an important fat-soluble antioxidant in the body and plays a role in protecting the cells from ROS attack in operation with the other enzymic antioxidants. They play an important role in lipid peroxidation and in the regulation of the SOD activity by maintaining low levels of

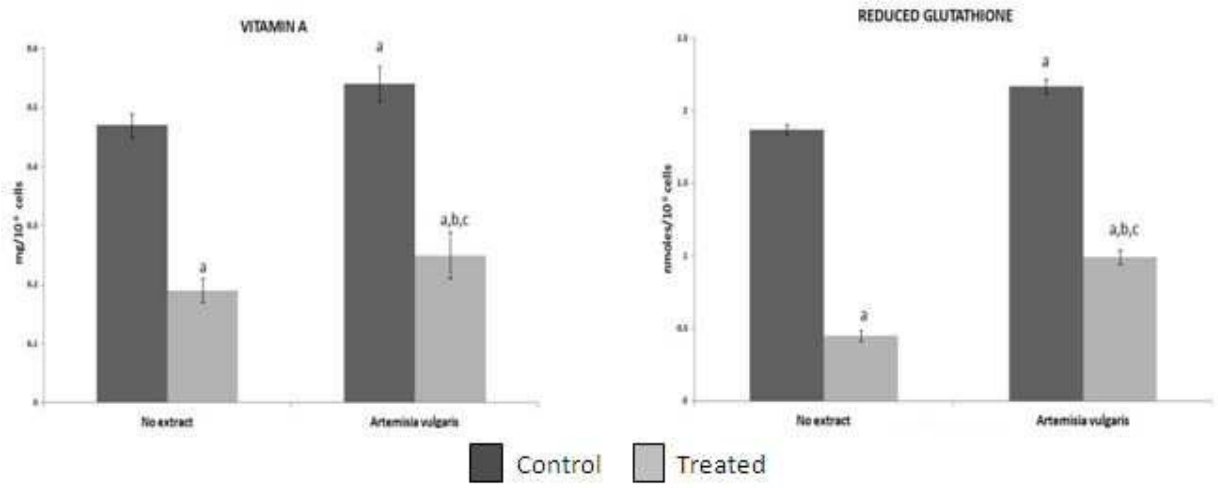
superoxide in the cells [28]. Carotenoids are the precursors for Vitamin A and have physiological functions in the elimination of excess energy, scavengers of free radicals and quenchers of singlet oxygen [29]. GSH, a non-protein cellular thiol plays a regulatory role in cell proliferation along with GPx in conjugation. The electrophilic moieties involved in cancer initiation is effectively scavenged by GSH dependent enzymes [30]. The levels of Vitamin C, E, A and reduced glutathione were estimated in the chick embryo fibroblasts exposed to an oxidant (H_2O_2) in the presence and absence of the leaf extract. The levels of the non-enzymic antioxidants are shown graphically in the Figures 3 and 4. Upon exposure to the oxidant there was a significant reduction in the levels but showed an increase in the presence of the methanolic extract of *A. vulgaris* leaves. Animals with DMBA induced hamster buccal pouch carcinogenesis when treated with the methanolic extract of the *Pergularia daemia* aerial parts showed an increase in the Vitamin C, E and GSH levels clearing depicting their antioxidant property [31]. Similarly, the effect of the methanolic extracts of *Alstoria scholaris* showed an improvement in the antioxidant status when assessed on non-enzymatic antioxidants like vitamin A, E, C *in vitro* in breast cancer tissues [32]. The antioxidants showed an increase in the levels in the presence of the methanolic extract of *Verbascum thapus* leaves when compared to the gentamin treated rats [33]. Aqueous extract of *Aloe vera* showed to increase the SOD and GSH levels in the streptozotocin induced diabetic rats when compared to the control [34].

Figure 3
Effect of Artemisia vulgaris leaf extract on the levels of vitamin C and E in primary chick embryo fibroblasts exposed to H_2O_2 in vitro



The values are mean ± S.D of triplicates
a – Statistically significant (P<0.001) compared to untreated control
b – Statistically significant (P<0.001) compared to H_2O_2 alone treated group
c – Statistically significant (P<0.001) compared to the respective plant extract treated group

Figure 4
Effect of Artemisia vulgaris leaf extract on the levels of vitamin A and reduced glutathione in primary chick embryo fibroblasts exposed to H_2O_2 in vitro



The values are mean ± S.D of triplicates
a – Statistically significant (P<0.001) compared to untreated control
b – Statistically significant (P<0.001) compared to H_2O_2 alone treated group
c – Statistically significant (P<0.001) compared to the respective plant extract treated group

The observations that both enzymic and non-enzymic levels increased in the presence of *A. vulgaris* leaf extract in primary chick embryo fibroblasts, during oxidative stress, gains a lot of significance in view of the previous studies. Artemisinin and dihydroartemisinin have been shown to be an active component of the Asteraceae genus [35, 36]. *In silico* studies has shown the compounds to effectively dock against few major apoptotic and cancer proteins (results communicated). Studies on the molecular mechanism has been carried out, but was considered beyond the scope of this paper. Thus, the results show an elevation in the antioxidant status of the chick embryo fibroblasts when exposed to oxidative stress *in vitro* in the presence of *Artemisia vulgaris* leaf extract and helps in protecting the cells. This *in vivo* simulated *in vitro* model can suggest the same in the intact system too.

CONCLUSION

The outcome of the present study highlights the protective effect of the leaf extract of *Artemisia vulgaris* on non-transformed cells, chick embryo fibroblasts suggesting the presence of certain active principle compounds rendering the antioxidant effect against oxidative stress induced *in vitro*. Further, use of the extract on transformed cells will throw light on the use of the plant in diseases caused by free radicals.

Conflict of interest
Conflict of interest declared none.

REFERENCES

1. Halliwell B, Free radicals and antioxidants: updating a personal view. Nutr Rew, 70 (5): 257 – 265, (2012).
2. Boskabadi H, Moeini M, Tara F, Tavallaie S, Saber H, Netaji R, Hosseini G, Mostafavi-Toroghi H, Ferns GAA, Ghayour-Mobarhan M, Determination of prooxidant-antioxidant balance during uncomplicated pregnancy using a rapid assay. J Med Biochem, 32 (3): 1 – 6, (2013).
3. Paschos A, Pandya R, Duivenvoorden WCM, Pinthus JH, Oxidative stress in prostate cancer: changing research concept towards a novel paradigm for prevention and therapeutics. Prostate cancer and prostatic diseases, doi:10.1038/pcan.2013.13, (2013).

4. McGrowder DA, Anderson-Jackson L, Crawford TV, Biochemical evaluation of oxidative stress in type 1 diabetes, Chapter 9, InTech, , 223 – 248, (2013).
5. Sen S, Chakraborty R, Sridhar C, Reddy YSR, Biplab D, Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect, Int. J. Pharma. Sci. Rev. Res., 3: 91 – 100, (2010).
6. Chopra RN, Nayar SL, Chopra IC, Glossory of Indian Medicinal Plants, CSIR, New Delhi, 26: (1956).
7. Haniya AK, Padma PR, Free radical scavenging activity of *Artemisia vulgaris* L. leaf extracts, World J. Pharm. Pharm. Sci., 2: 6381 – 6390, (2013).
8. Sharmila K, Padma PR, Anticancer activity of *Artemisia vulgaris* on hepatocellular carcinoma (HepG2) cells, Int. J. Pharm. Pharm. Sci., 5: 479 – 483, (2013).
9. Kiruthika B, Manickam SD, Padma PR, *Zea mays* leaf extracts protect primary chick embryo fibroblast cells from apoptosis induced by hydrogen peroxide *in vitro*, J. Pharm. Res., 6:638 – 646, (2013).
10. Kakkar P, Das B, Viswanathan PN, A modified spectrophotometric assay of superoxide dismutase. Ind J Biochem Biophys, 21 (2): 130 – 162, (1984).
11. Luck H, In: methods in enzymatic analysis, II Edn., Bergmeyer Academic Press, New York, 805, (1974).
12. Reddy KP, Subhani SM, Khan PA, Kumar KB, Effect of light and benzyladenine on dark treated graving rice (*Oryza sativa*) leaves - changes in peroxidase activity. Plant Cell Physiol, 26: 987 – 994, (1995).
13. Habig WH, Pabst MJ, Jakoby M, Glutathione transferase: A first enzymatic step in mercapturic acid formation. J Biol Chem, 249 (22): 7130 – 7139, (1974).
14. Roe JH, Keuther CA, The determination of ascorbic acid in whole blood and urine through 2,4-dinitrophenylhydrazine derivative dehydro ascorbic acid. J Biol Chem, 147: 399 – 407, (1943).
15. Rosenberg HR, Chemistry and physiology of the vitamins. Interscience Publisher, New York, 452 – 453, (1992).
16. Bayfield RF, Cole ER, Colorimetric estimation of vitamin A with trichloroacetic acid. Meth. Enzymol., 67: 189 – 195, (1980).
17. Moron MS, Depierre JN, Mannervik VC, Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochem Biophys Acta. 582: 67 – 68, (1979).
18. Hayakawa N, Asayama S, Noda Y, Shimizu T, Kawakami H, Pharmaceutical effect of manganese porphyrins on manganese superoxide dismutase deficient mice. Mol Pharm, 9: 2956 – 2959, (2012).
19. Noichri Y, Chalghoum A, Chkioua L, Baudin B, Ernez S, Ferchichi S, Miled A, Low erythrocyte catalase enzyme activity is correlated with high serum total homocysteine levels in Tunisian patients with acute myocardial infarction. Diagnostic Pathology, 8: 68, doi:10.1186/1746-1596-8-68, (2013).
20. He SY, Zhang GC, Yu YQ, Li RG, Yang QR, Effects of vacuum cooling on the enzymatic antioxidant system of cherry and inhibition of surface-borne pathogens. Int J Refrig, doi:10.1016/j.ijrefrig.2013.05.018, (2013).
21. Manevich Y, Hutchens S, Tew KD, Townsend DM, Allelic variants of glutathione S-tranferase P1-1 differentially mediate the peroxidase function of peroxiredoxin VI and alter membrane lipid peroxidation. Free Rad Biol Med, 54: 62 – 70, (2013).
22. Barua CC, Begum SA, Talukdar A, Roy JD, Buragohain B, Pathak DC, Sarma DK, Bora RS, Gupta A, Influence of *Alternanthera brasiliana* (L.) Kuntze on altered antioxidant enzyme prolife during cutaneous wound ehaling in immunocompromised rats. Int. Scholarly Res. Network, doi:10.5402/2012/948792, (2012).
23. Balasubramanian K, Padma PR, Protection of precision cut-goat liver slices by *Zea mays* leaf extracts from hydrogen peroxide induced oxidative stress *in vitro*. Int J Pharm Bio Sci, 3 (4): 201 – 210, (2012).
24. Olorunnisola OS, Afolayan AJ, *In vivo* antioxidant and biochemical evaluation of

Sphenocentrum jollyanum leaf extract in *P. berghei* infected mice. Pak J Pharm Sci, 26 (3): 445 – 450, (2013).
25. Dolai N, Karmaka I, Kumar RBS, Bala A, Mazumder UK, Haldar PK, Antitumor potential of *Castanopolis indica* (Roxb. Ex Lindl.) A. DC. leaf extract against Ehrlich's ascites carcinoma cell. Ind J Exp Biol, 50: 359 – 365, (2012).
26. Swathi D, Prasad KVSRG, Sree PR, Jalaiah M, Antiulcer and antioxidant activity of ethanolic extract of *C. hirsutus* against ethanol induced gastric ulcer in Albino rats. Int J Pharm Bio Sci, 4(3): 74 – 78, (2013).
27. Al-azzawie HF, Umran A, Hyader NH, Oxidative stress, antioxidant status and DNA damage in mercury exposure workers. Br J Pharmacol Toxicol, 4 (3): 80 – 88, (2013).
28. Caetano AC, da Veiga LF, capaldi FR, de Alencar SM, Azevedo RA, Bezerra RMN, The antioxidant response of the liver of male Swiss mice raised on a AIN 93 or commercial diet. BMC Physiol, 13: 3, doi: 10.1186/1472-6793-13-3, (2013).
29. Amengual J, Lobo GP, Golczak M, Li HNM, Klimova T, Hoppel CL, Wyss A, Palczewski K, von Lintig J, A mitochondrial enzyme degrades carotenoids and protects against oxidative stress. The FASEB Journal, 25, 948 – 959, (2011).
30. Rosa MS, Felipe L, Juan CM, Radical decisions in cancer: redox control of cell growth and death. Cancers, 4: 442 – 474, (2012).
31. Karthishwaran K, Mirunalini S, Assessment of the antioxidant potential of *Pergularia daemia* (Forsk.) extract *in vitro* and *in vivo* experiments on hamster buccal pouch carcinogenesis. Asian Pacific J Tropical Disease, 5509 – 5516, (2012).
32. Surendra SP, Jayanthi G, Smitha KR, *In vitro* evaluation of the anticancer effect of methanolic extract of *Alstonia scholaris* leaves on mammary carcinoma. J Appl Pharm Sci, 2 (5): 142 – 149, (2012).
33. Pal H, Kumar T, Kaki H, *In vitro* antioxidant and renoprotective potential of methanolic extract of *Verbascum thapus* leaf in rats. Pelagia Research Library, 4 (2): 14 – 23, (2013).
34. Mohapatra S, Pradhan S, Rath B, Tripathy S, Antioxidant properties of *Aloe vera* in streptozotocin induced diabetic rats. Int J Pharm Bio Sci, 4 (3): 187 – 191, (2013).
35. McGovern PE, Christofidou-Solomidou M, Wang W, Dukes F, Davidson T, El-Deiry WS, Anticancer activity of botanical compounds in ancient fermented beverages (review), Int. J. Oncol., 37: 5 – 14, (2010).
36. Kong R, Jia G, Cheng Z, Wang Y, Mu M, Wang S, Pan S, Gao Y, Jiang H, Dong D, Sun B, Dihydroartemisinin enhances Apo2L/TRAIL-mediated apoptosis in pancreatic cancer cells vis ROS-mediated up-regulation of death receptor 5, PLoS ONE, 7: doi; 10. 1371/journal.pone. 0037222, (2012).