The Bioscan 8(3): 1035-1038, 2013 (Supplement on Toxicology)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

TOXOLOGICAL REMEDIATION OF OXIDATIVE STRESS BY LEAF EXTRACT OF *SPHAGNETICOLA TRILOBATA* IN LYMPHOCYTE OF *ORYCTOLAGUS CUNICULUS* L.

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KEYWORDS
Oxidative stress
S. trilobata
Antioxidant

Received on :
05.05.2013

Accepted on :
27.07.2013

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ABSTRACT
Free radicals, generated by the various endogenous systems of our body, in the form of ROS and RNS adversely alter lipid, protein and DNA constitution by inducing damage in cells. This is due to toxic lipid peroxidation in biomembranes causing disruption in natural defense system of the body. Consequently, the condition of oxidative stress develops that lead to a number of diseases. Antioxidants neutralize the adverse effects of free radicals, thus preventing the conditions of pathogenecity. The naturally occurring antioxidant constituents of many plants act as radical scavengers and inhibitors of the toxicological process of oxidation. The antioxidant activity of leaf extract of *Sphagneticola trilobata* was evaluated in the present study, against hydrogen peroxide induced oxidative damage in lymphocytes of *Oryctolagus cuniculus* L. (Rabbit). Incubated lymphocytes with H_2O_2 for 2h, increased toxicity due to lipid peroxidation affecting the activities of MDA (4.69 ± 0.04), SOD (1.60 ± 0.03), GSH (2.48 ± 0.03), CAT (2.87 ± 0.03) and GPx(5.60 ± 0.10). Treatment with the extract of leaves of *Sphagneticola trilobata* for 18h was found to effectively inhibit (MDA 1.09 ± 0.03) or reduce lipid peroxidation and positively stimulate the activities of the antioxidant enzymes, SOD (3.39 ± 0.03), CAT (5.69 ± 0.05) and GPx (10.56 ± 0.02) and glutathione (5.26 ± 0.03) significantly usually with 20μL extract. The results, thus, indicated the free radical scavenging action of leaf extract of *Sphagneticola trilobata* to minimize toxicity.

INTRODUCTION

The unique antioxidant defense system of organisms and human beings has the remarkable ability to fight against all the odds and imbalances created by cellular oxidations and generation of free radicals and finally the toxic effect. The oxidative damage resulting due to the critical and unfavorable balance between free radical generation and antioxidant defenses lead to oxidative stress (Rock *et al.,* 1996). Oxidative stress, thus arising, is associated with damage to a wide range of molecular damage to a wide range of molecular species including lipids, proteins and nucleic acids (Mc Cord *et al.,* 2000). This leads to a number of toxicity induced disorders and diseases.

The inhaled oxygen (O_2), an essentiality for life (Mohammed *et al.,* 2004), often have toxic effects due to the formation of ROS such as O_2^-, H_2O_2 and OH (Bagchi *et al.,* 1998). The antioxidants present in the defense systems are in the form of substances which are present in low concentration compared to that of any oxidizable substrate, that would significantly, delay or prevent the oxidation of that substrate (Halliwell *et al.,* 1995). Antioxdants can decrease oxidative stress and toxic effect by direct scavenging of ROS or by inhibiting cellular damage through breaking the chain propagation reactions of lipid peroxidation (Irshad *et al.*, 2002; Niki, 1991). The various enzymes, vitamins, minerals and compounds of high and low molecular weights provide the spine to the antioxidant defense system (Blois, 1958; Prior *et al.,* 1998; Mantena *et al.,* 2003; Khanam *et al.,* 2004; Shirwalkar *et al.,* 2004).

The awareness towards health and fitness has brought in the trend of routinely use of antioxidants through food, tonics, food supplements and medicines. The use of synthetic antioxidants has led to the increasing risk factors for many dreadful diseases, due to which these are avoided nowadays (Papas, 1999). The preference has shifted towards the use of antioxidants present in medicinal and dietary plants (Brown *et al.,* 1998). This has linked to the olden times, when plants formed the basis of traditional medicine systems, and has, since then provided mankind with new remedies.

The plant of *Sphagneticola trilobata* (Asteraceae), is commonly used in many traditional medicines. Its leaf extract is, effectively used to cure cold and flu, to clear the placenta post-birth, and for treatment of hepatitis and inflammation (Xuesong, 2006). The wedeolites of leaves are used as antimalarial, anti-cancerous, antimutagenic, antifungal and analgesic compounds (Maldini *et al.,* 2009). The present paper emphasizes on the antioxidant property of leaf extract of *Sphagneticola trilobata,* investigated against hydrogen peroxide induced oxidative damage and toxic effect in blood lymphocytes of *Oryctolagus cuniculus* L.

MATERIALS AND METHODS

Plant extract

The plant (*Sphagneticola trilobata*) was collected from the

nearby open grounds / fields of Durg and Bhilai city of Chhattisgarh, India. The leaf extract was prepared in 59% alcohol by Soxhlet Extraction apparatus, and used for the present investigation, the solvent used was Methanol.

***In vitro* study**

Blood sample was collected from *Oryctolagus cuniculus* L. (Rabbit) following density gradient method in heparinized sterilized tube. The sample was washed in phosphate buffer saline and then placed in Dulbercoo's Modified Eagles Media (DMEM) alongwith 10% fetal calf serum for culture. Cells were cultured in our Tissue Culture Lab. in a humified CO_2 incubator at 37°C temperature and 5% CO_2 for 18 hours. After incubation, cells were resuspended in fresh media and exposed to oxidative stress with 100μ M H_2O_2 for 2 hours (Sohi *et al.,* 2003).

The experimentation constituted the cultured lymphocytes which were divided into five groups, and in each group five samples were considered.

Group I: Only lymphocytes

Group II: Lymphocytes + 100μM H_2O_2 for 2 hours.

Group III: H_2O_2 treated lymphocytes pretreated with 5μL/10000 cells of *Sphagneticola trilobata* leaf extract.

Group IV: H_2O_2 treated lymphocytes pretreated with 10μL/ 10000 cells of *Sphagneticola trilobata* leaf extract.

Group V: H_2O_2 treated lymphocytes pretreated with 20μL/ 10000 cells of *Sphagneticola trilobata* leaf extract.

The cells were collected, washed twice in icecold phosphate buffer and used for biochemical assay.

Biochemical studies

To evaluate the effect of leaf extract of *Sphagneticola trilobata* on oxidative stress caused by H_2O_2 to cultured lymphocytes, the following biochemical parameters were analyzed:

· Lipid peroxidation in terms of Malondialdehyde (MDA) was determined by thioborbituric acid reaction following Okhawa *et al.*, 1979.

Reduced Glutathione (GSH) following Moron *et al.,* 1979.

Superoxide dismutase (SOD) following Misra *et al.,* 1972.

Catalase (CAT) following Bergmeyer *et al.*, 1974.

Glutathione peroxide GPx following Rotruck *et al.,* 1973.

Statistical analysis

The collected data for all parameters were statistically validated by ANOVA.

RESULTS

The H_2O_2 treated lymphocytes showed significant increase in malondialdehyde (MDA) activity as compared to control. But in the three sets of experiments, a gradual successful recovery ($p < 0.05$) was observed with increasing concentration (5μL/ 10 μL / 20μL) of leaf extract of *Sphagneticola trilobata* as compared to H_2O_2 treated cells. (Table 1)

Reduced glutathione (GSH) showed decrease in its activity in H_2O_2 treated cells as compared to control. With the gradual increase of treatment of leaf extract (5μL / 10 μL / 20 μL) in the three sets of experiments that followed, the glutathione activities increased and got restored ($P < 0.05$) as compared to H_2O_2 treated cells. (Table 1)

The activities of superoxide dismutase, catalase and glutathione peroxidase decreased distinctly ($P < 0.05$) when treated with H_2O_2 in comparison to control cells, but, the treatment with the leaf extracts in increasing concentrations (5μL / 10 μL / 20 μL) resulted in the increased activities of all the enzymatic parameters ($P < 0.05$). (Table 1).

DISCUSSION

The oxidative stress and related toxicological impact directly alter major macromolecules (DNA, carbohydrates, Protein) of the body. The impairment so caused brings about the passiveness in the antioxidant defense system. The abrupt fluctuating level of antioxidants gives rise to various pathological conditions.

The various kinds of antioxidants have been recognized to counter the toxicity of free radicals generated by oxidative stress. SOD, CAT, GTx, glutathione reductase and some minerals like Se, Mn, Cu and Zn are considered as antioxidants of first line of defense. GSH, Vitamin C, uric acid, albumin, bilirubin, Vitamin E, carotenoid and flavonoids etc. are considered as antioxidants of second line of defense. As a third line defensive antioxidants, complex group of enzymes are being considered which plays significant role in repair of damaged DNA, damaged protein, oxidized lipids and peroxides. It is also supposed that third line antioxidants effectively stop chain propagation of peroxyl lipid radicals

Table 1: Effect of leaf extract of *Sphagneticola trilobata* on various enzymatic activities of H_2O_2 induced lymphocytes of *Oryctolagus cuniculus* L.

Parameters	Group I(Control)	Group II(H_2O_2 treated)	Group III(5μL of PLE and H_2O_2)	Group IV(10μL of PLE and H_2O_2)	Group V(20μL of PLE and H_2O_2)
Lipid peroxides (in mole MDA/ mg protein)(MDA)	0.08 ± 0.02	4.69 ± 0.04*	3.90 ± 0.03#	3.27 ± 0.04#	1.09 ± 0.03#
Reduced Glutathione (μ moles / mg protein) (GSH)	6.29 ± 0.04	2.48 ± 0.03*	3.42 ± 0.03#	3.88 ± 0.02#	5.26 ± 0.03#
Superoxide dismutase (Units/mg protein) (SOD)	3.86 ± 0.02	1.60 ± 0.03*	1.82 ± 0.03#	2.23 ± 0.02#	3.39 ± 0.03#
Catalase μ moles of H_2O_2 consumed / min. / mg protein (CAT)	6.21 ± 0.03	2.87 ± 0.03*	3.18 ± 0.02#	3.69 ± 0.03#	5.69 ± 0.05#
Glutathione peroxide (μg of glutathione utilized / min. / mg proteins) (GPx)	10.84 ± 0.04	5.60 ± 0.10*	7.69 ± 0.06#	8.32 ± 0.03#	10.56 ± 0.02#

PLE – Plant leaf extract ; * compared with control; # compared with H_2O_2

(Gupta *et al.,* 2006).

Lymphocytes contain a diversified redox and free radical scavenging system (Robinson *et al.*, 1993; Halliwell *et al.,* 1989) and are extensively screened for exposure of various toxicants (Petushkova *et al.,* 1996). The hydrogen peroxide having capability to cross cell membrane and to stimulate hydroxyl radical formation (Yu, 1994; Halliwell *et al.,* 1986), so in the present study hydrogen peroxide was used to induce oxidative stress in the lymphocytes of *Oryctolagus cuniculus* L. For present study, it was supposed that leaf extract of *Spagneticola trilobata* can also cross the membrane *in vitro* and can also neutralize the free radicals and check the lipid peroxidation in H_2O_2 stressed cell. The hypothesis was tested in the present investigation. We found increased activity of malondialdehyde due to activation of lipid peroxidation in H_2O_2 treated lymphocytes. It is inferred that during the process, excessive free radicals was generated and attacked on fatty acid components of membrane lipid resulting in membrane rigidity and receptor realignment (Kaplan *et al.,* 1995; Nawak *et al.,* 2002). The concept of increased free radical formation was also found supported by decrease in reduced glutathione in H_2O_2 treated lymphocytes (Spiesky *et al.,* 1985; Demir *et al.,* 2003).

In the present study we found marked disturbances in GSH metabolism supported by decreased activity of superoxide dismutase, catalase and glutathione peroxidase in H_2O_2 treated lymphocytes of *Oractolagus cuniculus*.

The literature regarding property of *Sphagneticola trilobata* (*Wedelia trilobata*) is not available except Govindappa *et al.,* 2011. They studied antioxidant activity of leaf, stem and flower crude ethanolic extract, measured the ability to scavenge DPPH free radicals and compared with standard / ascorbic acid and butylated hydroxytouene (BHT). They observed that ethanol extract of leaf had higher activity than that of stem and flower.

But evaluation of antioxidant property of plant by assay of enzymatic parameters in *in vitro* cultured and H_2O_2 induced lymphocyte was not studied previously. Although, Kumar, in 2009 has reported similar finding in H_2O_2 induced lymphocyte of human by leaf extract of *Adiantum capillus veneris*. The concept was also supported by Koke *et al.,* 1990 and Hashim *et al.,* 2005.

Our conclusion is the leaf extract of *Sphagneticola trilobata* showed ameliorative effect against toxicity caused by free radicals with increased dose.

REFERENCES

Rock, C. L., Jacob, R. A. and Bowen, P. E. 1996. Update on biological characteristics of the antioxidant micronutrients – Vitamin C, Vitamin E and the carotenoids. *J. Am. Diet. Assoc.* **96(7):** 693-702.

Mc Cord, J. M. 2000. The evolution of free radicals and oxidative stress. *Am. J. Med.* **108**: 652-9.

Mohammed, A. A. and Ibrahim, A. A. 2004. Pathological roles of reactive oxygen species and their defence mechanis. *Saudi. Pharm. J.* **12:** 1-18.

Bagchi, K. and Puri, S. 1998. Free radicals and antioxidants in health and disease. *East Mediterranean Health J.* **4:** 350-60.

Halliwell, B. 1995. How to characterize an antioxidant - An update. *Biochem Soc. Symp.* **61:** 73-101.

Irshad, M. and Chaudhari, P. S. 2002. Oxidant – Antioxidant system : Role and significance in human body, *Indian J. Exp. Biol.* **40:** 1233-1239.

Niki, E. 1993. Antioxidant defenses in eukaryptic cells. In: Poli G, Albano E, Dianzani mU, editors. Free radicals: From basic science to medicine. Basel, Switzerland: *Birkhauser Verlag.* pp. 365-73.

Prior, R. L., Cao, G., Martin, A., Sofic, E., Mc Ewen, J., O'Brien, C., lischner, N., Eblenfeldt, M., Kalt, W., Krewer, G. and Mainland, C. M. 1998. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity and variety of Vaccinium species *J. Agaric. Food Chem.* **46(7)**: 2686-2693.

Khanam, S., Shivprasad, H. N. and Devi, K. 2004. *In vitro* antioxidant screening models: a review. Indian *J. Pharm. Edu.* **38(4):** 180-183.

Blois, M. S. 1958. Antioxidant determinations by the use of a stable free radical. *Nature.* **181:** 1199-1200.

Shirwaikar, A., Rajendran, K. and Kumar, C. D. 2004. *In vitro* antioxidant studies of *Annona squamosa* Linn. *Leaves, Indian J. Exp. Biol.* **42:** 803-807.

Matena, S. K., Jagdish Badduri, S. R., Siripurapu, K. B. and Unikrishnan, M. K. 2003. In vitro evaluation of antioxidant properties of Cocos nucifera Linn. water. *Food/Nahrung.* **2:** 126-131.

Papas, A. M. 1999. Diet and antioxidant status. *Food Chem. Toxicol.* **37:** 999-1007.

Brown, J. E. and Rice-Evan, C. A. 1998. Luteolin-rich Artichoke extract protects low density lipoprotein from oxidation in vitro. *Free Radic Res.* **29:** 247-255.

Xuesong, H. U. A. N. G. 2006. Simultaneous Determination of Trilobolide-6-O-Isobutyrates A and B in *Wedelia trilobata* by Gas Chromatography. **24(5):** 499-502.

Maldini, M., Sosa, S. and Montoro, P. 2009. Screening of the tropical anti-inflammatory ativity of the bark of Acacia cornigera Willdenow, Byrsonime crassifolia Kunth, Sweetia panamensis Yakovler and the leaves of Sphagneticola trilobata Hitchcock, *J. Ethnopharmacology.* **122(3):** 430-433.

Sohi, K. K., Mittal, N., Hundal, M. K. and Khanduja, K. L. 2003. Gallic acid, an antioxidant, exhibits antiapoptotic potential in normal human lymphocytes: A Bcl-2 independent mechanism. *J. Nutr. Sci. Vitaminol.* **49(4)**: 221-227.

Okhawa, H., Ohishi, N. and Yogi, K. 1979. Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. *Analyt. Biochem.* **95:** 351-354.

Moron, M. S., Depierre, J. W. and Mannervik, B. 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochem. Biophys. Acta.* **582:** 67-68.

Misra, H. P. and Fridovich, I. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.* **247(10):** 3170-3175.

Bergmeyer, H. V., Gowehu, K. and Grassel, M. 1974. Methods of Enzyme Analysis, Acad. *Press, New York.* p. 438.

Rotruck, J. T., Pope, A. L., Ganther, H., Swanson, A. B., Hafeman, D. G. and Hocksira, W. G. 1973. Selenium: biochemical role as a component of glutathione peroxidase. *Science.* **179**: 588-590.

Gupta, V. K. and Sharma, S. K. 2006. Plants as natural antioxidants. *Nat. prod. Rad.* **5(4)**: 326-334.

Robinson, M. K., Rodrick, M. C., Jacobs, D. O., Rounds, J. D., Collins, K. H., Saporoschetz, I. B., Mannick, J. A. and Wilmore, D. W. 1993. IB, Mannick JA, Wilmore DW. Glutathione depletion in rats impairs T-cell and macrophage immune function. *Arch. Surg.* **126**: 29-35.

Halliwell, B. G. and Gutteridge, M. C. 1989. Free Radicals in Biology and Medicine. , *2nd Ed, Oxford Science Pub. Univ. Press.*

Petuchkova, N. A. and Bachmanova, G. I. and Archakov, A. I. 1996. 7-Alkoxyphenoxazone and 7-Alkoxycoumarin O-Dealkylation By Human Peripheral Blood Lymphocytes, In Vitro And Molecular Toxicology: J. Basic appl. *Res., Toxicol*. **9:** 291-295.

Yu, B. P. 1994. Cellular defenses against damage from reactive oxygen species. *Physiol. Rev*. **74(1):** 139-162.

Halliwell, B. G. and Gutteridge, M. C. 1986. Oxygen free radicals and iron in relation to biology and medicine: Some problems and concepts *J. Arch. Biochem. Biophys*. **246(2)**: 501-514.

Kaplan, P., Recay, P., lehotsky, J. and Mexesova, V. 1995. Changes in fluidity of brain endoplasmic reticulum membranes by oxygen free radicals: a protective effect of stobadine, alpha tocopherol acetate and butylated hydroxyl toluene. *Neurochem. Res.* **20:** 815-820.

Nowak, E., Wyrwicz, G., Dabrowski, Z., Smolenski, O. and Spodarek K. 2002. Rheological and biochemical examination ofred blood cells after treatment with differentpharmacological and toxic substances. *Clin. Hemorheol. Microcirl*. **26:** 91-97.

Spiesky, H., Macdonald, A., Giles, G., Orreyo, H. and Israel, Y. 1985. Increased loss and decreased synthesis of hepatic glutathione after acute ethanol administration. *Turnover studies Biochem. J.* **225(3):** 565-572.

Demir, M., Konkoglu, D., Kabasakal, l., Yelije, D. I. K., Ergen. K. and Ahmed, S. 2003. The effects of exposure of 60 Co on the oxidant/ antioxidant status among radiation victims. *J. Environ. Radioact*. **64(1):** 19-25.

Govindappa, M., Naga Sravya, S., Poojashri, M. N., Sadananda, T. S. and Chandrappa, C. P. 2011. Antimicrobial, antioxidant and *in vitro* anti-inflammatory activity of ethanol extract and active phytochemical screening of *Wedelia trilobata* (L.) Hitchc. *J. Pharmacog. Phytothe*. **3(3):** 43-51.

Kumar, A. 2009. Antioxidant effect of Adiantum capillus veneris Linn. On Human lymphocyte: an *in vitro* study. *J. cell and Tissue Research*. **9(2):** 1899-1902.

Koke, J. R. Christodoulides, N. J., Chudej, L. L. and Bittar, N. 1990. Exogenous superoxide dismutase and catalase promote recovery of function in isolated rat heart after regional ischemia and may be transported from capillaries into myocytes. *Mol. Cell. Biochem*. **96(2):** 97-106.

Hashim, M. S., Lincy, S., Remya, V., Jeeva, M. and Anila, L. 2005**. Effect of polyphenolic compounds from Coriandrum sativum on H_2O_2-induced oxidative stress in human lymphocytes. *Food Chem.*** 92(4): **653-660.**