Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol.2, Issue, 1, pp. 001-007, January, 2011

ISSN: 0975-833X

RESEARCH ARTICLE

ANTIOXIDANT EFFECT OF *Ocimum gratissiumum* LINN. LEAF AGAINST CARBON TETRACHLORIDE (CCl_4)-INDUCED OXIDATIVE STRESS IN WISTAR ALBINO RATS

Okonkwo, C.J. and Njoku, O.U.

Lipid and Lipoprotein Research Unit, Department of Biochemistry,
University of Nigeria, Nsukka.

ARTICLE INFO

Article History:

Received 15th, November, 2010
Received in revised form
13th, December, 2010
Accepted 25th December, 2010
Published online 5th January, 2011

Key words:

Ocimum gratissimum;
Ethanol leaf extract;
CCl_4-induced oxidative stress;
Antioxidant effect;
Antioxidant nutrients.

ABSTRACT

The leaves of *Ocimum gratissimum* (OG) were assayed for its phytochemical, vitamin and essential mineral content. Also, ethanol leaf extract of OG was investigated for antioxidant activity against carbon tetrachloride (CCl_4)-induced oxidative stress in Wistar albino rats. Plasma lipid peroxidation inhibition and activities of the antioxidant enzymes, superoxide dismutase (SOD) and glutathaine peroxidase (GPx) were used as antioxidant indices, while Vitamin E (1000 IU, d-alpha-tocopheryl acetate soft-gel capsule) served as reference. Phytochemical analysis revealed the presence of Flavonoids, terpenoids, alkaloids and glycosides. The mean levels of zinc (Zn), manganese (Mn), copper (Cu) and iron (Fe) were 1.54, 6.16, 4.76 and 21.16 ppm, respectively, while those of beta-carotene, vitamins C and E were 0.222 ± 0.02, 1.59 ± 0.15 and 19.96 ± 0.69 mg/100g. Result of the bioassay demonstrated that the CCl_4-intoxicated rats were subjected to oxidative stress as indicated by low activities of their antioxidant enzymes; SOD (54.0 ± 8 U/l), GPx (67254 ± 1790 U/l) and high plasma lipid peroxidation index (61.86 ± 4.09 µg/ml) when compared with normal control rats value of 202.5 ± 23 U/l, 81497 ± 1012 U/l and 48.78 ± 8.04 µg/ml, respectively. The ethanol extract of *O. gratissimum* leaf reversed this situation as shown by the increased activity of the antioxidant enzymes and lipid peroxidation inhibition. The results indicated that ethanol extract of *O. gratissium* leaf significantly protected the rats from CCl_4-induced oxidative stress. Furthermore, its antioxidant effect is dose-related.

INTRODUCTION

Antioxidant agents of natural origin have attracted special interest because they can protect the human body from free radicals (Osawa *et al*., 1990) and are apparently associated with fewer side effects. Moreover, knowledge and application of such potential antioxidant activities in reducing oxidative stress *in vivo* has prompted many investigators to search for potent and cost-effective

***Corresponding author:** edubch@yahoo.com

antioxidants from various plant sources (Shyur *et al*., 2005). These research studies have contributed to new and renewed public interests worldwide in herbal medicines, health foods and nutritional supplements (Shyur *et al*., 2005). Many minor components of foods such as secondary plant metabolites have been shown to alter biological processes, which may reduce the risk of chronic disease in human (Nwanjo *et al*., 2006). It is believed that phytochemicals may be effective in combating or preventing diseases due to their antioxidant potential (Farombi *et al*., 1998).

Ocimum gratissimum Linn., a popular medicinal plant and spice in Africa and Asia (Effraim *et al*., 2003), belongs to the family Lamiaceae, genus *ocimum* and species *gratissimum*. The plant is found throughout the tropics and subtropics and its greatest variability occurs in tropical Africa and India (Aruna and Sivaramakrishina). The morphology of the plant has been documented (Wagner *et al*., 1999). The leaves and stems of the plant are very rich in essential oils (Sulistiarini *et al*., 1999). The whole plant and the essential oils are widely used in traditional medicine for the treatment of bacterial and fungal infections, diarrhoea, diabetes, inflammatory conditions etc. (Akinmoladum *et al*., 2007). Akinmoladun *et al*. (2007) also reported the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity and reductive potential of the methanol leaf extract of *O. gratissimum*. However, the antioxidant potentials of the plant in animal model have not been documented. Hence, the present study was aimed at evaluating the effect of ethanol extract leaf of *O. gratissimum* on lipid peroxidation and antioxidant enzymes (SOD and GPx) activities in male Wistar albino rats subjected to CCl_4-induced oxidative stress.

MATERIALS AND METHOD

Chemicals and Reagents

Commercial test kits used in this study were products of RANDOX Laboratories Ltd, Crumlin, United Kingdom. All other Chemicals and reagents used were of analytical grade.

Plant Materials

Fresh leaves of the plant were collected from Nsukka, Enugu State, Nigeria and authenticated by Mr. P. O. Ugwuozo of the Department of Botany, University of Nigeria, Nsukka (UNN). A voucher specimen was deposited at the herbarium unit of the Department for reference.

Animals

Male Wistar albino rats of 8 to 12 weeks old weighing 150-200 g were used for the investigation. The rats were obtained from the animal house of the Faculty of Biological Sciences, UNN. The animals were kept at an average temperature of 30^{o}C and a 12 h light/dark cycle. They were fed with standard pellet diet (product of Bendel Feed Nigeria Ltd.) obtained commercially. All animals had free access to food and water *ad libitum* and were acclimatized to laboratory condition for a week before commencement of experiment.

Extraction Procedure

Fresh leaves of the plant were cleaned and air-dried at ambient temperature for two weeks. The dried leaves were pulverised to a coarse powder using a mechanical grinder. A weighed quantity, 200 g, of the coarse powder was extracted by cold maceration in absolute ethanol for 48 h. The extraction mixture was filtered with Whatman No. 1 filter paper. The filtrate was concentrated *in vacuo* at less than 40^{o}C in a rotary evaporator to obtain a dark-green semi-solid mass. The extractive yield (%) was estimated gravimetrically. The extract was stored +4^{o}C until used.

Phytochemical Screening

Chemical tests were carried out on the extract for the qualitative determination of phytochemical constituents as described by Harbone (1998).

Vitamins and Mineral Estimation

The prevailing levels of antioxidant vitamins and minerals in OG leaf was determined by standard method (AOAC, 2006)

CCl_4-Induced Oxidative Stress

Eighteen rats were randomly distributed into six groups of three rats each. Treatment lasted for a period of four days. Group 1 (normal control, NC) received normal rat diet with free access to water *ad libitum*. Group 2 (solvent control, SO) received 5.0 ml/kg body weight (b.w.) of 5% Tween 80 per orally (p.o.). Group 3 (negative control, NE) were similarly treated as group 1. Group 4 (standard control, SC) received 4.08 ml/kg b.w. of Vitamin E (1000 IU, d-alpha-tocopheryl acetate soft-gel capsule) p.o. Groups 5 and 6 (test groups) received 500 and 1000 mg/kg b.w. of the extract p.o., respectively. On the third day, CCl_4 was administered by intraperitoneal injection at a dose of 0.75 ml/kg b.w. (in double the volume of olive oil which served as the vehicle) to all rats except the rats in group I. After treatment for 4 days, the animals were fasted for 24 h but had free access to water. Blood was collected via ocular puncture into heparin bottles on day 5. A portion of the blood samples were centrifuged and the plasma collected for lipid peroxidation estimation while the remaining heparinized whole blood samples were reserved for enzyme assay.

Lipid Peroxidation Estimation

Lipid peroxidation in the plasma fraction was estimated spectrophotometrically by assessing the levels of thiobarbituric acid reactive species (TBARS) and the lipid peroxidation product, malondialdehyde (MDA) following the method described by Wallin *et al*. (1993).

Superoxide Dismutase Assay

A volume, 0.5 ml, of heparinized whole blood sample was centrifuged for 10 min at 3000 rpm and the plasma aspirated off. The erythrocytes were washed four times with 3 ml of normal saline. The washed erythrocytes were haemolysed by making up the volume to 2.0 ml with cold distilled water. The haemolysate was left to stand at $+4^oC$ for 15 min after which a 50 fold dilution was prepared using 0.01 mol/l phosphate buffer, pH 7.0. The diluted haemolysate was used to determine superoxide dismutase (SOD) activity according to the method outlined by Suttle (1986). RANDOX (RANSOD) superoxide dismutase test kit (Cat^{118} No. SD 125) was used.

Glutathione Peroxidase Assay

Glutathione peroxidase activity was determined by the method of Paglia and Valentine (1967). RANDOX (RANSEL) glutathione peroxidase test kit (Cat. No. RS. 504) was used.

Statistical Analysis

The results were expressed as mean ± SD. Means were compared for statistically significant difference using one-way analysis of variance (ANOVA). Differences between means were considered significant at $P<0.05$ in all cases.

RESULTS

The percent extract yield was 5.86% w/w (with respect to the powdered material). Phytochemical analysis of the extract revealed the presence of bioactive substances listed in Table 1. Table 2 shows some antioxidant nutrient content of the leaves. The mean levels of beta-carotene, vitamins C and E were 0.222 ± 0.02, 1.59 ± 0.15 and 19.96 ± 0.69 mg/100 g respectively, while those of zinc (Zn), manganese (Mn), copper (Cu) and iron (Fe) were 1.54 ± 0.05, 6.16 ± 0.10, 4.76 ± 0.36 and 21.16 ± 0.52 mg/100 g, respectively. Fig. 1 reveals that lipid peroxidation level was highest in the CCl_4-treated group (negative control). The extract showed a dose-dependent lipid peroxidation inhibiting property. At both doses of the extract tested, the levels of lipid peroxidation were lower than that of the standard control. Furthermore, the lipid peroxidation inhibition expressed by the upper dose (1000 mg/kg) of the extract, in relation to the normal and standard control groups, was significant at $P<0.05$. Fig 2 shows that SOD activity reduced significantly ($P<0.05$) in CCl_4-administered rats, when compared with normal control. Treatment with 500 mg/kg of the extract increased enzyme activity, though insignificantly ($P<0.05$). However, treatment with 1000 mg/kg of extract raised SOD activity significantly ($P<0.05$), as compared to CCl_4-treated group, restoring the enzyme activity to almost normalcy.

Table 1. Classes of phytocompounds in the extract

Class of phytocompound	*Ocimum Gratissimum Leaf*
Tannins	ND
Flavonoids	+
Saponins	+
Terpenoids	+++
Steroids	ND
Alkaloids	+
Tropane alkaloids	ND
Morphine alkaloids	ND
Glycosides	++
Cardiac glycosides	ND
Glycosides with deoxy-sugar	+
Glycosides with steroidal nucleus	ND
Resins	+

Key: +++ = High; ++ = Moderate; + = Low; ND = Not Detected

Table 2. Levels of some antioxidant nutrients in OG leaf

Antioxidant nutrient	Level (mg/100 g)
Vitamin C	1.59 ± 0.15
Vitamin E	19.96 ± 0.69
Beta-carotene	0.222 ± 0.09
Manganese (Mn)	6.16 ± 0.10
Zinc (Zn)	1.54 ± 0.05
Iron (Fe)	21.16 ± 0.52
Copper (Cu)	4.76 ± 0.36

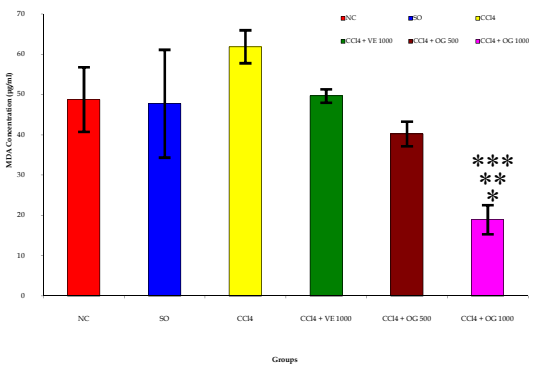

Fig 1. Effect of the extract on the lipid peroxidation level of rats subjected to CCl_4-induced oxidative stress. * P<0.05 compared with NC, ** P<0.05 compared with NE, *** P<0.05 compared with SC.

In Fig. 3 the unprotected animals (negative control) showed the lowest glutathione peroxidase (GPx) activity. Treatment with both doses of extract restored GPx activity slightly above the normal and standard controls.

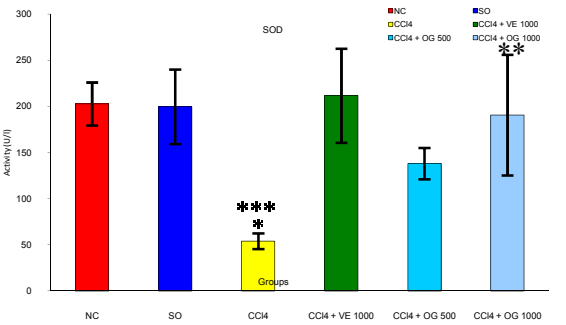

Fig. 2. Effect of the extract on SOD activity of rats subjected to CCl_4-induced oxidative stress. * P<0.05 compared to NC, ** P<0.05 compared to NE, *** P<0.05 compared to SC.

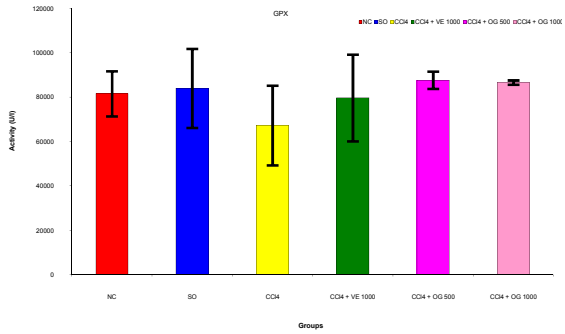

Fig. 3. Effect of the extract on GPx activity of rats subjected to CCl_4-induced oxidative stress.

DISCUSSION

Phytochemical analysis revealed the presence of such bioactive substances like flavonoids, terpenoids, tannins, saponins, alkaloids and resins (Tabble 1). The medicinal properties of these component phytocompounds have been reported (Banso and Adeyemo, 2007). These phytochemicals are most likely responsible for the antimicrobial activity of the plant. Flavonoids and terpenoids are well established antioxidants. Flavonoids have been reported to possess antioxidant activity approximately 50 times that of vitamins C and E (Friedli, 2005). The antioxidant effects of the extract may be attributed to the presence of flavonoids and terpenoids in addition to other factors.

The result in Table 2 indicated that fresh leaves of the plants are good sources of antioxidant vitamins. The protective action of fruits and vegetables has been attributed, partly, to the presence of antioxidant vitamins including ascorbic acid, α-tocopherol and beta-carotene (Grivetti and Ogle, 2000). It has been reported that supplementing with 500 mg/day of vitamin C for two weeks increased the glutathione concentration of the blood by 50 per cent (Johnson *et al.,* 1993). Glutathione is one of the body's most important natural antioxidants. Vitamin E appears to be the most important lipid-soluble antioxidant protecting membranes from lipid peroxidation by acting as a chain-breaking antioxidant (Traber and Atkinson, 2007). It also limits the oxidation of LDL cholesterol and may help prevent or delay the development of atherosclerosis and/or coronary heart disease (CHD) (Jialal and Fuller, 1995). Beta carotene is the precursor of vitamin A, which is necessary for the production and resynthesis of rhodopsin. High levels of beta-carotene intake have been correlated with lower risk of lung cancer, coronary heart disease, stroke and age-related eye disease (Johnson, 1993).

In addition to their numerous biological roles, Zn, Mn, Fe and Cu also serve as co-factors in certain biochemical reactions including those involving antioxidant enzymes. Iron serves as a co-factor for the enzyme catalase, a primary antioxidant that detoxifies hydrogen peroxide by dismutation to water and oxygen. Similarly, Fe, Mn, Cu and Zn are all vital co-factors of the different forms of SOD found in plants and animals. It is therefore suggested that these minerals contribute to the antioxidant effect of the extract probably by boosting the levels of antioxidant enzymes such as SOD and catalase.

Lipid peroxidation induced by CCl_4 is a commonly used experimental animal model for studying oxidative injury in biological systems (Recknagel and Glende, 1989). In the present study, excessive lipid peroxidation in CCl_4-intoxicated rats (negative control) demonstrated the capacity of CCl_4, at the dose used, to induce lipid peroxidation in animals. However, treatment with the ethanol leaf extract of *O. gratissimum* inhibited the CCl_4-induced lipid peroxidation in a dose-dependent manner. This indicated the ability of the extract to protect against lipid peroxidation, a major mechanism of cell injury in organisms exposed to oxidative stress. The vehicle, Tween 80, had no antioxidant effect as the values of the solvent control (SO) were very close to those of the normal control (NC) for all parameters investigated.

SOD is an en zyme that catalyses the dismutation of superoxide anion radical ($\cdot O_2^-$) to H_2O_2 and molecular (O_2) (Zelko *et al*., 2002). Its action therefore protects the biological integrity of cells and tissues against the harmful effects of ROS (Otitoju and Onwurah, 2006). GPx scavenges H_2O_2 and other peroxides. This selenium-containing enzyme, requiring glutathione co-factor, is a major antioxidant enzyme. Glutathione, a tripeptide with a thiol group is found in plants and animals in both its reduced and dimeric forms. In the reduced (monomeric) form, glutathione is a powerful endogenous antioxidant, protecting biological systems from degenerative damage associated with ageing and oxidative stress (Nelson and Cox, 2003). Hence, oxidative imbalance will result in[211] depletion of glutathione, thereby lowering GPx activity. SOD and GPx are for primary defense against reactive oxygen metabolites (Mahdi, 2002). Such metabolites have been implicated in the damage brought about by ionizing radiation, as well as in the effects of cytostatic compounds (Marklund *et al*., 1982).

The decreased activity of antioxidant enzymes along with elevated lipid peroxidation levels in CCl_4-induced rats could probably be associated with oxidative stress and/or decreased antioxidant defense potential (Mahdi *et al*., 1996). Similar decreases in the activity of antioxidant enzymes and elevated level of lpid peroxidation in rats subjected to oxidative stress had been reported (Arulselvan and Subramanian, 2008). The reversal in their activity following treatment with the extract may be due to decreased oxidative load. The extract may also act by either directly scavenging the reactive oxygen metabolites, due to the presence of various antioxidant compounds (Gupta *et al*., 2002), or by increasing the synthesis of antioxidant molecules/enzymes.

Conclusion

The capacity of the ethanol leaf extract of *O. gratissimum* to inhibit lipid peroxidation and restore the activities of the antioxidant enzymes to almost normal levels have demonstrated that it possesses potent antioxidant properties. However, further studies are required to isolate and elucidate the structure of the active principles since there is need to develop new potent and cost-effective antioxidants from plant sources.

Acknowledgements

The authors are thankful to Dr. E. P. Joshua and Mr. U. Nwachi, Department of Biochemistry, University of Nigeria, Nsukka for technical assistance; and Mr. P. O. Ugwuozo, Department of Botany of same university for authentication of the plant materials.

REFERENCES

Akinmoladun, A.C., Ibukun, E.O., Afor, E., Obuotor, E.M. and Farombi E.O. 2007. Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum. Science Research Essay*, 2(5): 163-166.

Arulselvan, P. and Subramanian, S. 2008. Ultrastructural and biochemical abnormalities in the liver of streptozotocin-diabetic rats: Protective effects of *Murraya koenigii. Journal of Pharmacology and Toxicology,* **3**(3): 190-202.

Aruna, K. and Sivaramakrishina, V.M. 1990. Plants as protective agents against cancer. *Indian Journal of Experimental Biology*, 28(11): 108-111.

Association of official Analytical Chemists (AOAC) International. 2006. Official methods of analysis. 18th ed. Washington D. C. pp 220-240.

Banso, A. and Adeyemo, S.O. 2007. Evaluation of antibacterial properties of tannins isolated from *Dichrostachys cinerea.* African Journal of Biotechnology, 6(15): 1785-1787.

Effraim, K.D., Jacks, T.W. and Sodipo, O.A. 2003. Histopathological studies on the toxicity of *Ocimum gratissimum* leaf extract on some organs of rabbit. African Journal of Biomedical Research, 6(1): 21-25.

Farombi, E.O., Nwamkwo, J.O. and Emerole, G.O. 1998. Effect of methanolic extract of browned yam flour diet on 7,12-Dimethylbenzanthracene (DMBA) and 3-ethylcholanthrene (3-MC) induced toxicity in the rat. Proceedings of Fedrated African Society of Biochemistry and Molecular Biology, 1: 5-10.

Friedli, G. 2005. Glycosides. Friedly Enterprises. *georgeslouis@friedli.com.*

Grivetti, L.E. and Ogle, B.M. 2000. Value of traditional foods in meeting macro- and micronutrients needs: the wild plant connection. *Nutrition Research and Reviews,* 13: 31-46.

Gupta, S.K., Prakash, J. and Srivastava, S. 2002. Validation of traditional claim of Tulsi, *Ocimum sanctum* Linn. as medicinal plant. *Indian Journal of Experimental Biology,* 40: 765-773.

Harborne, J.B. 1998. Phytochemical Methods. 3rd ed. London:Thompson Science. pp. 107-150.

Jialal, I. and Fuller, C.J. 1995. Effect of vitamin E, selenium and beta-carotene on LDL oxidation and atherosclerosis. *Canadian Journal of Cardiology,* 11: 97G-103G.

Johnson, E.J. 1993. Aging and carotene nutriture. Age 16: 59-66.

Johnson, C.S., Meyer, G.G., Srilakshmi J.C. 1993. Vitamin C elevates red blood cell glutathione in healthy adults. *American Journal of Clinical Nutrition,* 58: 103-105.

Nelson, D.L. and Cox, M.M. 2003. Leninger principles of Biochemistry. 4th ed. New York: W. H. Freeman and Company. pp. 721-722.

Mahdi, A.A. 2002. Free radicals and other antioxdants. In: Singh SP, (ed). A textbook of Biochemistry. 3rd ed. New Delhi: CBS Publishers and Distributors. pp. 545-555.

Mahdi, A.A., Singh R. and Singh, R.K. 1996. Role of reactive oxygen species and antioxidants in human disease. In: Rai V, Nait ML, Monoharacharry C, (eds). An Overview of Perpectives in Biology. Ranpur: World Laser Graphics, pp. 55-70.

Marklund, S.L., Westman, N.G., Lundgren, E. and Ross, G. 1982. Copper and Zinc containing superoxide dimutase, catalase, and glutathione peroxidase in normal and neoplastic human cell

lines and normal human tissues. *Cancer Research,* 42: 1955-1961.

Nwanjo, H.U. and Alumanah, E.O. 2005. Effect of aqueous extract of *Gongronema latifolium* leaf on some indices of liver function in rats. *Global Journal of Medical Sciences,* 4(1): 29-32.

Nwanjo, H.U., Okafor, M.C. and Oze, G.O. 2006. Anti-lipid peroxidative activity of *Gongronema latifolium* in streptozotocin-induced diabetic rats. *Nigerian Journal of Physiological Sciences,* 21(1-2): 61-65.

Osawa, T., Kavakishi, S. and Namiki, M. 1990. In: Kuroda Y, Shankal DM, Waters MD, (eds). Antimutagenesis and anticarcinogenesis mechanisms II. New York: Plenum, p. 139.

Otitoju, O. and Onwurah, I.N.E. 2006. Effects of Rambo insect powder on glutathione-S-transferase (GST) and superoxide dismutase (SOD) activity in rats. *Bio-Resource,* 4(1): 51-58.

Paglia, D. and Valentine, W. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine*, 70: 158-169.

Recknagel, R.O. and Glende, E.A. 1989. The carbon tetrachloride hepatotoxicity model: free radicals and calcium homeostasis. In: Miquel J, Quintanilha AT, Weber H, (eds). Handbook of free radicals and antioxidants in Biomedicine. vol. 3. Boca Raton: CRC Press. pp. 3-16.

Shyur, L.F., Tsung, J.H., Chen, J.H., Chiu, C.Y. and Lo, C.P. 2005. Antioxidant properties of extracts from medicinal plants popularly used in Taiwan. *International Journal of Applied Sciences and Engineering,* 3(3): 195-202.

Sulistiarini, D., Oyen, L.P.A. and Nguyen X.D. 1999. *Ocimum gratissimum* L. In: Plant Resources of South-East Asia. Bogar, Indonesia: Prosea Foundation. pp. 140-142.

Suttle, N.F. 1986. Copper deficiency in ruminants: recent developments. The Veterinary Record, 119: 519-522.

Traber, M.G. and Atkinson J. 2007. Vitamin E, antioxidants and nothing more. *Free Radical Biology and Medicine*, 43(1): 4-15.

Wagner, W.L., Herbst, D.R. and Sohmer, S.H. 1999. Manual of the flowering plants of Hawaii. Revised ed. Bernice, P. Bishop Museum special publication. University of Hawaii Press/Bishop Museum Press, Honolulu. p. 1919.

Wallin, B., Kosengreen, B., Shertzer, H.G. and Camejo, G. 1993. Lipoprotein oxidation and measurement of TBARS formation in a single microlitre plate; its use for evaluation of antioxidants. *Journal of Analytical Biochemistry*, 208: 10-15.

Zelko, I., Mariani, T. and Folz, R. 2002. Superoxide dismutase multigene family: a comparison of the Cu/Zn-SOD (SOD1), Mn-SOD (SOD2) and EC-SOD (SOD3) gene structures, evolution and expression. *Free Radical Biology and Medicine,* 33(3): 337-349.

****