Global Veterinaria 4 (3): 230-236, 2010
ISSN 1992-6197

Rosemary Extract Protect Against γ-irradiation Induced Clastogenicity

[1]Zeinab E. Hanafy and [2]Sohair A. Hassan

[1]Department of Zoology, Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt
[2]Department of Medicinal Chemistry, National Research Centre, Giza, Egypt

Abstract: Rosemary (*Rosmarinus officinalis*) is widely accepted as one of the medicinal herb with the highest antioxidant activity. The present study aimed to examine the radioprotective potentiality and efficacy of Rosemary against damage induced by whole body γ-irradiattion. This study was performed on four groups of female rats, i.e. control group, Rosemary group, γ-irradiated group and Rosemary and γ-irradiated group. In each group, cytogenetical studies,chromosome aberrations and micronucleus test, have been done using bone marrow cells. DAN fragmentation assay have been done using liver cells. Results reveled that the chromosome aberration assay in bone marrow cells and micronuclei cells pointed out a significant difference between rats received Rosemary post irradiation and γ-irradiated rats. Also, there was a significant increase in DNA fragmentation in γ-irradiated group. When Rosemary was used after irradiation the percentage of DNA fragmentation was significantly decreased. It was concluded that Rosemary *(Rosmarinus officinalis)* extract was effective in the protection against clastogenic effects of radiation due to their higher content of different antioxidant constitutes and its ability to scavenge free radicals generated by radiation.

Key words: *Rosmarinus officinalis* · DAN fragmentation · Micronucleus test · Chromosome · Rats

INTRODUCTION

Ionizing radiation is known to affect somatic and germ cells, leading to mutation, cell death, malformation and cancer. Interactions of ionizing radiation with living cells cause a variety of changes, whose damage intensity depend, fundamentally on the absorbed dose, type of radiation, conditions of irradiations and intrinsic radiosensitivity of cell [1].

A considerable number of researches have been carried out in the field of chemical radioprotection during the last few decades; however, no safe and ideal synthetic radioprotectors are available to date. Recently, interest has generated in developing the potential botanical drugs for the amelioration of radiation effects. Plants and their products are well known to have an advantage over the synthetic compounds in terms of their potential low/no toxicity at the effective dose with minimum or no side effects [2-6]. However, the use of medicinal plants is characterized by lack of robust scientific evidence to support their use. Therefore, studies supporting or rejecting their role in the treatment of various health disorders are of great need [2].

Rosemary (*Rosmarinus officinalis*), belonging to the family Lamiaceae, is a common medicinal and aromatic plant, grown in many parts of the world. It is indigenous to Southern Europe, particularly on the dry rocky hills of the Mediterranean region. Rosemary is used as a culinary herb, a beverage drink, as well as in cosmetics. In folk medicine, it is used as analgesic, antirheumatic, carminative, diuretic, expectorant, anti-epileptic, anti-spasmodic in renal colic, dysmenorrhoea, for relief of respiratory disorders, improve human fertility and stimulate hair growth [7]. Rosemary has been shown to be safe in toxicity studies in animal models when added as an antioxidant to food [8].

Since time immemorial, the plant has been used traditionally by people for curing various health disorders around the world. Caribs of Guatemala use Rosemary to cure various human diseases [9]. Rosemary (*Rosmarinus officinalis*) which is used in traditional Turkish folk medicine for the treatment of hyperglycaemia, is widely accepted as one of the medicinal herb with the highest antioxidant activity [10]. Thus, wide acceptability and diverse pharmacological and anti-oxidative properties of the plant stimulated us to evaluate the radio modulatory effect of *Rosmarinus officinalis* in female rats exposed to dose of 5 gray gamma radiation.

Corresponding Author: Zeinab E. Hanafy, Department of Zoology, Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt

MATERIALS AND METHODS

Taxonomic Description of the Plant: *Rosmarinus officinalis* is an ever green shrub growing to 1.5 m at a medium rate. The leaves of Rosemary are about 1 inch long, linear, revolute, dark green above and paler and glandular beneath, with camphoraceous aromatic odour. The scented hermaphrodite flowers are small and pale blue. Much of the active volatile principle resides in their calyces. There are various other varieties of the plant, but the green-leaved variety is the kind which is used medicinally [11] .

Preparation of Plant Extract: The plant *Rosmarinus officinalis* (family: Lamiaceae) dried leafs were obtained from a trusted traditional herbal market, Cairo, Egypt. The plant material was prepared by extracting 20 g of leaf with double boiled distilled water by rinsing for 36 hrs at room temperature. Filtered solution of the extract was obtained by filtration through a simple mesh. An approximate yield of 22 % extract (w/w) was obtained.

The required dose for treatment was injected intraperitoneally (at a dose of 0.5ml /kg body wt.). Optimum dose 1000 mg/kg body wt. [12].

Irradiation: The cobalt teletherapy unit (Co-60) at the Irradiation Tecnology Center, Dokki Giza, was used for irradiation. Unanaesthetized animals were restrained in well-ventilated perspex boxes and exposed to gamma radiation (i.e. 5 Gy) at a distance (SSD) of 80 cm from the source at a dose rate of 0.85 Gy/min.

Animals: Forty female rats (100-120g) were obtained from the house of the experimental animals, the National Research Center, Cairo, Egypt. The animals were kept under normal healthy laboratory conditions inbred colony were used in the present study. Animals were provided with standard rat feed (procured from Animal Nutrient Co., Cairo) and water *ad libitum* and were maintained under controlled conditions of temperature and light (Light: dark, 10 hrs: 14 hrs.). 5 animals were housed in a polypropylene cage with locally procured paddy husk (Oryza sativa) as bedding throughout the experiment. Tetracycline-containing water (0.13 mg/ml) was provided once a fortnight and was given as a preventive measure against infections. Animal care and handling were performed according to the guidelines set by the World Health Organization (WHO), Geneva, Switzerland and the ETC (Ethical Committee National Reaserch Center), Cairo, Egypt.

Experimental Design: Rats were divided into four groups to carry out the experimental study. These groups were as follows:

Group-I: control

Group-II: was given *Rosmarinus officinalis* extract intraperitoneally (1000 mg/kg body wt) daily for 2 weeks

Group-III: was exposed to 5 Gy gamma rays.

Group-IV: was given *Rosmarinus officinalis* extract intraperitoneally (1000 mg/kg body wt) 24 hour after irradiation for 2 weeks. All these groups were observed daily up for two weeks for any sign of sickness, behavioral toxicity and mortality. Rats were autopsied after two weeks post γ-irradiation exposure with and without Rosemary treatment.

Bone marrow was collected for cytogenetic analysis (Chromosome preparation and micronucleus test) and the tissue samples from livers were collected for DNA fragmentation.

Chromosome Preparation: The animals were sacrificed and bone marrow was collected. Chromosome preparation from bone marrow cells of female rat was prepared according to the procedure of Yosida and Amano [13].

The micronucleus test: from control and all the treated groups were extracted; smear preparations were made by using fetal calf serum according to the method of Salamone *et al.* [14].

DNA Fragmentation Assay: The method of DNA fragmentation was carried out according to Burton and Perandones *et al.* [15,16].

Statistical Analysis: The obtained data were subjected to analysis of PRIMER Ver 5.0 according to Bary-curtis Similarity Inedex [17].

RESULTS

Chromosomal Aberrations: The current study revealed that exposure to radiation resulted in highly increased frequency of aberrations in bone marrow cells as compared with the control group or those treated with *Rosmarinus officinalis* extract alone or in combination with radiation (Table 1). These aberrations included chromosomal gaps, breaks, rings, deletions, centromeric attenuation and endomitosis.The frequency of aberrant

Table 1: Effect of γ-irradiation with and without rosemary on chromosome aberrations and aberrant cells in bone marrow cells of female rat after two weeks (Mean)

	Chromosomal aberrations						
	Structural					Numerical	
Groups	Gap	Break	Ring	Deletion	Centromeric attenuation	Endomitosis	Total chromosomal aberrations
Control	0.5	1		2.8	-----	2.6	6.8
Rosemary	1	1.6	1	3	2	6	14.4
Radiation	0.6	0.2	0.8	14	5	7	27.8
radiation + Rosemary	-----	1	1.2	5	2	5	14.2

Table 2: Effect of γ-irradiation with and without rosemary on micronuclei (MN) in bone marrow cells of female rat after two weeks

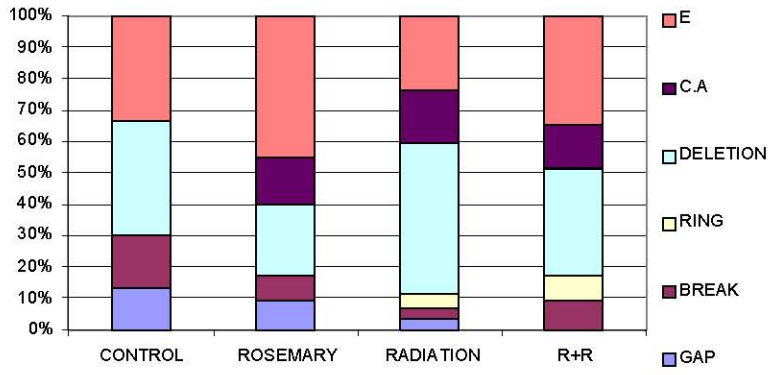
Groups	MN frequency	Mean
Control	11,12,10,12,14	13.8
Rosemary	12,16,15,13,16	14.4
Radiation	70,64,58, 55, 57	61.0
Radiation + Rosemary	39,32,31,35, 36	34.6

Table 3: Effect of γ-irradiation with and without rosemary on DNA fragmentation in liver cells of female rat after two weeks

Groups	DNA fragmentation %	%Change
Control	12.2	----
Rosemary	20.0	+ 7.8
Radiation	53.0	+ 40.8
Radiation + Rosemary	46.2	+ 34.0

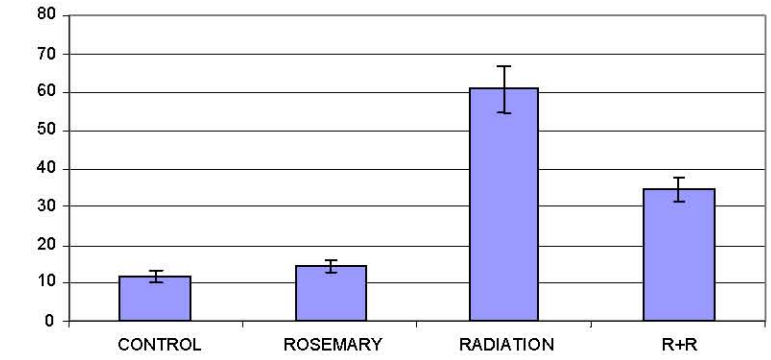
Table 4: Similarity between different groups of female rat after two weeks

Groups	control	Rosemary	Radiation	Radiation + Rosemary
control	0	0	0	----
Rosemary	76.1347	0	0	0
Radiation	36.11529	52.32085	0	0
Radiation + Rosemary	56.42458	75.21388	69.61967	0



C.A: Centromeric attenuation E: Endomitosis R+R: radiation+Rosemary

Fig. 1: Effect of γ-irradiation with and without rosemary on chromosome aberrations in bone marrow cells of female rat after two weeks



R+R: radiation +Rosemary

Fig. 2: Effect of γ-irradiation with and without rosemary on micronuclei (MN) in bone marrow cells of female rat after two weeks

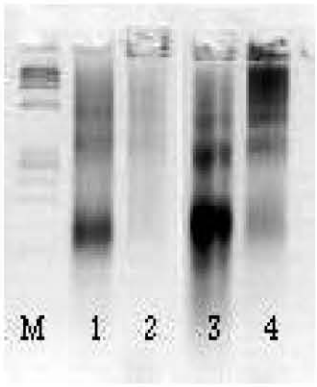
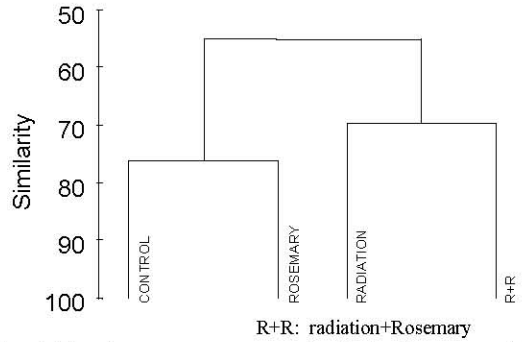


Fig. 3: Lane M: DNA molecular weight marker
Lane 1: control Lane 2: Rosemary
Lane 3: radiation Lane4: Rosemary after radiation



R+R: radiation+Rosemary

Fig. 4: Dendrogram represent similarity between deferent groups of female rat after two weeks

cells in the group of animal treated with *Rosmarinus officinalis* only slightly increased when compared to control. The combined treatment of *Rosmarinus officinalis* extract and radiation resulted a decrease in the number of total chromosomal aberrations in bone marrow cells resulted from radiation (Table 1, Fig.1).

Micronucleus Test: On analyzing the frequency of micronucleated cells in bone marrow cells (Table 2), it was found that radiation induced highly increase in the frequency of micronucleated cells. When Rosemary administrated after radiation, it decreased the formation of micronucleated cells.

DNA Fragmentation: The mean of DNA fragmentation showed slightly increase after treatment with *Rosmarinus officinalis* as compared to the control group. However, the mean of DNA fragmentation induced by radiation was highly increased. Administration of *Rosmarinus officinalis* decreased DNA fragmentation induced by radiation (Table 3 and Fig 3).

DISCUSSION

The development of agents that protect tissues against radiation damage (i.e., radioprotective agents) is currently the subject of intense research on at least two broad accounts. First, radiotherapy remains one of the most widely used treatments for cancer. Irradiation-induced DNA damage can halt tumor cell proliferation, but collateral radiation damage to surrounding tissues is always a concern. Accordingly, there is a need to develop drugs that will protect healthy cells while leaving malignant cells susceptible and ideally, even sensitized to radiation therapy. An additional impetus for research is the need to counteract occupational risks and terrorist threats of radiation exposure.

In the present study, irradiation showed a significant increase in frequency of chromosomal aberrations, micronuclei cells and DNA fragmentation. Similar to these observations, it was reported that ionising-radiation induces a wide range of molecular lesions in mammalian

cells that can lead to diverse cellular responses such as cell in activation, chromosomal rearrangements and mutations, eventually resulting in cancer and hereditary diseases [18]. In addition, Vijayalaxmi *et al.* [19] found that cells exposed to γ-irradiation exhibited a pronounced increase in chromosome damage. DNA damage occurs either by direct ionisation or indirectly through generation of free radicals that attack DNA, resulting in single-strand breaks and oxidative damage to sugar and base residues [20], which may later be converted into DNA double strand breaks. It is understood that the un-repaired double strand breaks will contribute to chromosomal aberrations [18,21]. Genotoxic effects of ionizing radiation are also mediated through formation of free radicals and reactive oxygen species that additionally harm DNA [22, 23].

Radiation induced damage to normal tissues can be partially reduced by the use of radioprotectors that reduce the damaging effects of radiation, including radiation-induced lethality [24-26]. Various workers have investigated the potential application of radioprotective chemicals in the event of planned and unplanned exposure (i.e., clinical oncology, radiation site cleanup, military scenarios, radiological terrorism, radiation accidents, etc.) [27-29].

Taking into account the limited successes of these chemical compounds, the need to identify naturals, effective and nontoxic substances of easy disponibility and with radioprotector abilities is justified. This makes possible to foresee promising strategies for protecting accidentally, occupationally or even therapeutically exposed individuals against ionizing radiation damage.

More important, *Rosmarinus officinalis* extract was not toxic and slightly increase in chromosomal aberration, micronuclei cells and DNA fragmentation. In this regards, Schuler [7] observed that the extract of *Rosmarinus officinalis* has been shown to be safe in toxicity studies in animal models when added as an antioxidant to food. *Rosmarinus officinalis* extract did not induce statistically significant increase in the average numbers of micronucleus or chromosome aberrations in bone marrow cells of Wistar rats [30, 31].

The present data revealed that Rosemary *(Rosmarinus officinalis)* extract showed protective effect against γ-radiation induced chromosomal aberration, micronuclei cells and DNA fragmentation. Furtado *etal.* [32] showed treatment with different concentrations of rosmarinic acid combined with doxorubicin DXR revealed a significant reduction in the frequency of micronuclei compared to animals treated with doxorubicin (DXR) only. Minnunni *et al.* [33] demonstrated that rosemary extract was antimutagenic in the Ames tester strain TA102. Singletary *et al.* [34] reported that the addition of Rosemary extracts to the diet as a 1% supplement by weight decrease the frequency of carcinogen-DNA adduct formation in rats. Andrade *et al.* [35] The V79 cells cultures were treated with different concentrations of rosmarinic acid in combination with doxorubicin (DXR). The results showed that rosmarinic acid (RA) exerted significantly reduced the frequency of micronuclei and the extent of DNA damage induced by DXR at the three concentrations tested.

Rosemary has been found to contain certain antioxidative and free radical scavenging activity [36]. Antioxidant properties of Rosemary are attributed not only to a single compound, but also to a synergistic action of several components. Clinical studies also support that Rosemary is an excellent antioxidant, having antimicrobial, anti-mutagenic, radioprotective and chemopreventive properties [7,12,37,38-41].

In conclusion, radiation was found to induce clastogenic effects in rat through the induction of oxidative stress and DNA modification. It increased the frequencies of chromosomal aberrations in bone marrow cells. Rosemary *(Rosmarinus officinalis)* extract was effective in the protection against these clastogenic effects of radiation due to their higher content of different antioxidant constitutes and its ability to scavenge free radicals generated by radiation.

REFERENCES

1. Hall, E.J., 2000. "Radiobiology for the radiologist", 5th ed. J.B. Lippincott, Philadelphia.
2. Ammon, H.P. and M.A. Wahl, 1991. Pharmacology of Curcuma longa. Planta Medica, 57: 1-7.
3. Scarterzzimi, P. and E. Speroni, 2000. Review of some plants of Indian traditional medicine with antioxidant activity. J. Ethnopharmacol., 71: 23-43.
4. Weiss, J.F. and M.R. Landauer, 2003. Protection against ionizing radiation by antioxidant nutrients and phytochemicals. Toxicol., 189: 1-20.
5. Coleman, C.N., H.B. Stone, J.E. Moulder and T.C. Pellmar, 2004. Modulation of radiation injury. Science, 304: 693-694.
6. Sarkar, F.H. and Y. Li, 2004. The role of isoflavones in cancer chemoprevention. Frontiers in Bioscience, 9: 2714-2724.
7. Al-sereiti, M.R., K.M. Abu-amer and P. Sen, 1999. Pharmacology of Rosemary (Rosmarinus officinalis Linn.) and its therapeutic potentials. Indian J. Experimental Biol., 37: 124-130.

8. Schuler, P., 1990. Natural antioxidants exploited commercially. In Food Antioxidants; B.J.F. Elsevier Applied Science, London.; Chapter-4.
9. Giron, L.M., V. Freire, A. Alonzo and A. Caceres, 1991. Ethnobotanical survey of the medicinal flora used by the Caribs of Guatemala. J. Ethnopharmacol., 34: 173-187.
10. Bakırel, T., U. Bakırel, O.Ü. Kele°, S.G. Ülgen and H. Yardibi, 2008. In vivo assessment of antidiabetic and antioxidant activities of rosemary (*Rosmarinus officinalis)* in alloxan-diabetic rabbits. J. Ethnopharmacol., 116: 64-73.
11. Grieve, M., 1971. A Modern Herbal: The Medicinal, Culinary, Cosmetic and Economic Properties, Cultivation and Folk-Lore of Herbs, Grasses, Fungi, Shrubs and Trees with their Modern Scientific Uses. Dover Publications, Inc. New York.
12. Sancheti, G. and P.K. Goyal, 2007a. Prevention of radiation induced hematological alterations by medicinal plant Rosmarinus officinalis, in Mice. African J. Alternative and Complementary Medicine, 4: 165-172.
13. Yosida, T.M. and K. Amano, 1965. Autosomal polymorphism in laboratory breed and wild norway rats. Rattus norveicus found in Misima. Chromosoma, 16: 658-667.
14. Salamone, M.F., J.A. Heddle, E. Stuart and A. Katz, 1980. Towards and improved micronucleus test: Studies on 3 model agents mitomycinc, cyclophosphamide and dimethylbenznthracene. Mutation Research, 44: 347-356.
15. Burton, K., 1956. A study of the condition and mechanisms of the diphenylamine reaction for the estimation of deoxyribonucleic acid. Biochem. J., 62: 315-323.
16. Perandones, C.E., V.A. Illera, D. Peckham, L.L. Stunz and R.F. Ashman, 1993. Regulation of apoptosis in vitro in mature murine spleen T-cells. J. Immunol., 151: 3521-3529.
17. Bary-curtis Similarity Inedex: PRIMER Ver, 5.0.
18. Satish Rao, B.S., R. Shanbhoge, D. Upadhya, G.C. Jagetia, S.K. Adiga, P. Kumar, K. Guruprasad and P. Gayathri, 2006. Antioxidant, anticlastogenic and radioprotective effect of *Coleus aromaticus* on Chinese hamster fibroblast cells (V79) exposed to gamma radiation. *Mutagenesis,* 21: 237-242.
19. Vijayalaxmi, Reiter, R.J. and M.L. Meltz, 1995. Melatonin protects human blood lymphocytes from radiation-induced chromosome damage. Radiation Research, 346: 23-31
20. Breen, A.P. and J.A. Murphy, 1995. Reactions of oxyl radicals with DNA. Free Radical Biology Medicine, 18: 1033-1077.
21. Burdak-Rothkamm, S., K. Rothkamm, M. Folkard, G. Patel, P. Hone, D. Lloyd, L. Ainsbury and K.M. Prise, 2009. DNA and chromosomal damage in response to intermittent extremely low-frequency magnetic fields. Mutation Research, 672: 82-89.
22. Shirazi, A., G. Ghobadi and M. Ghazi-Khansari, 2007. A radiobiological review on melatonin: a novel radioprotector. J. Radiation Res., 48: 263-272.
23. Malcaraz, M.D., M.D. Cacevedo, P.H.D. Jcastillo, P.H.D. Obenavente-Garcia, P.H.D. Darmero, M.D. Vvicente and M.S.C. Mcanteras, 2009. Liposoluble antioxidants provide an effective radioprotective barrier. British J. Radiol., 82: 605-609.
24. Kumar, A., J. Ram, R.M. Samarth and M.K. umar 2003. Modulatory influence of *Adhatoda Vasica* Nees leaf extract against gamma irradiation in Swiss albino mice. Phytomedicine, 12: 285-293.
25. Stone, H.B., J.E. Moulder, Coleman, *et al.* 2003. Models for evaluating agents intended for the prophylaxis, mitigation and treatment of radiation injuries. Radiation Research, 162: 711-728.
26. Baliga, M.S., G.C. Jagetia, P. Venkatesh and J.N. Ullor, 2004. Radio protective effect of abana, a polyherbal drug following total body irradiation. British J. Radiol., 77: 1027-1035.
27. Mettler, F.A.J. and G.L. Volez, 2002. Major radiation exposure-What to expect and how to respond. New England J. Medicine, 346: 1554-1561.
28. Johnson, R.H., 2004. Dealing with the terror of nuclear terrorism. Health Physics, 87: 53-57.
29. Waselenko, J.K., W.F. MacVittie, Blakely, *et al.* 2004. Medical management of the acute radiation syndrome: Recommendations of the strategic National Stockpile Radiation Working group. Annals of Internal Medicine, 140: 1037-1051.
30. Gaiani, T.F., J.C.T. Carvalho, J.M.S.F. da Silva and E.L. Maistro, 2006. Absence of Clastogenic Effects of the Extract from Medicinal Plant *Rosmarinus officinalis* L. on Wistar Rat Bone Marrow Cells. Cytologia, 71: 101-106.
31. Aguilar, F., D. Boskou, D. Gott, S. Grilli, R. Guertler, K. Hulshof, J.C. Larsen, J.C. Leblanc, C. Leclercq, A. Mortensen, D. Parent-Massin, I. Pratt, I. Rietjens, P. Tobback, G. Speijers and F. Toldra, 2008. Use of rosemary extracts as a food additive. The EFSA Journal, 721: 1-29.

32. Furtado, M.A., L.C.F. de Almeida, R.A. Furtado, W.R. Cunha and D.C. Tavares, 2008. Antimutagenicity of rosmarinic acid in Swiss mice evaluated by the micronucleus assay. Mutation Research/Genetic Toxicol. Environ. Mutagenesis, 657: 150-154.
33. Minnunni, M., U. Wolleb, O. Mueller, A. Pfeifer and H.U. Aeschbacher, 1992. Natural antioxidants as inhibitors of oxygen species induced mutagenicity. Mutation Research, 269: 193-200.
34. Singletary, K., C. MacDonald and M. Wallig, 1996. Inhibition by rosemary and carnosol of 7,12-dimethyl-benz [a]anthracene (DMBA)-induced rat mammary tumorigenesis and in vivo DMBA-DNA adduct formation. Cancer Letter, 104: 43-48.
35. Andrade, R., F.R.R. de Araújo, F.A. Resende, W.R. Cunha and D.C. Tavares, 2009. Protective effect of rosmarinic acid on V79 cells evaluated by the micronucleus and comet assays. J. Appl. Toxicol. 2009; Accepted: 22 September 2009. Published Online: 21 Oct 2009.
36. Saito, Y., A. Shiga, Y. Yoshida, T. Furuhashi, Y. Fujita and E. Niki, 2004. Effects of a novel gaseous antioxidative system containing rosemary extract on the oxidation induced by nitrogen dioxide and ultraviolet radiation. Bioscience, Biotechnol. Biochem., 68: 781-786.
37. W.O.A., 1997. The Wealth of Asia. Council of Scientific and Industrial Research (CSIR) Publication, New Delhi, CD ROM.
38. Sancheti, G. and P.K. Goyal, 2006a. Modulatory influence of Rosemarinus officinalis on DMBA-induced skin tumorigenesis in mice. Asian Pacific J. Cancer Prevention, 7: 331-335.
39. Sancheti, G. and P.K. Goyal, 2006b. Effect of Rosmarinus officinalis in modulating the 7, 12-dimethylbenz (a)anthracene induced skin tumorigenesis in mice. Phytotherapy Research, 20: 981-986.
40. Sancheti, G. and P.K. Goyal, 2007b. Role of Rosemary leaf extract against various doses of gamma radiation. Tree for Life J., 2: 2-7.
41. Georgantelis, D., I. Ambrosiadis, P. Katikou, G. Blekas and S.A. Georgakis, 2007. Effect of rosemary extract, chitosan and α-tocopherol on microbiological parameters and lipid oxidation of fresh pork sausages stored at 4 °C. Meat Science, 76: 172-181.