ejpmr, 2017,5(02), 323-330

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

INVESTIGATION ON THE ANTI-GENOTOXIC EFFECT OF *OCIMUM SANCTUM* IN FLUORIDE INDUCED MICRONUCLEI.

Srilatha Kambam[2*], David Banji[1], Otilia J.F. Banji[1] and Surekha Gonepally[2]

[1]Department of Pharmacology, Nalanda College of Pharmacy, Cherlapally, Nalgonda-508001, Telangana, India.
[2]Department of Pharmaceutical Chemistry, St Marys College of Pharmacy, Secunderabad, Telangana, India.

***Corresponding Author: Srilatha Kambam**

Department of Pharmacology, St Marys College of Pharmacy, Secunderabad, Telangana, India.

Article Received on 01/01/2017 **Article Revised on 21/12/2017** **Article Accepted on 11/01/2018**

ABSTRACT

The effect of various doses, viz. 100 mg/kg, 400 mg/kg, 800 mg/kg of body weight of ethyl acetate fraction of *Ocimum sanctum* (ETOAC) was studied in the fluoride induced micronucleated polychromatic (MNPCE) and normochromatic erythrocytes in mice bone marrow and peripheral blood. The treatment of mice with various doses of ETOAC before treatment with sodium fluoride resulted in significant decline in the frequency of MNPCE and MNNCE. The significant reduction in MNPCE and MNNCE was observed for 800 mg/kg bodyweight accompanied by the decrease in oxidative stress and better antioxidant capacity. The time dependent study was also carried out with 100 mg/kg for 3 days and 7 days in which the treatment for 7 days was found to be significant. The ETOAC was found to exhibit reduction in the formation of lipid peroxides. The highest FRAP value was obtained with 800 mg/ kg of ETOAC and similar effects were observed with 100 mg/kg of ETOAC for 7 days treatment. Our study demonstrates that ETOAC is able to protect fluoride induced micronuclei in mice bone marrow, peripheral blood and oxidative stress. The presence of flavonoids and tannins may be responsible for this protective action.

KEYWORDS: Fluoride, *Ocimum sanctum*, Micronuclei, Oxidative stress.

1. INTRODUCTION

17 out of 21 districts of united Andhra Pradesh, a state in southern India are affected with fluorosis. Nalgonda, a district in Telangana is deeply afflicted with fluoride related diseases, as the fluoride levels range from 2 to 7 mg/L in ground water. Large population is affected with fluorosis in this district alone.[1] Prevalence of endemic fluorosis can significantly impair human health. Excess fluoride intake causes fluorosis, which is an endemic public health problem in 22 nations across the globe including India.[2] The main sources of fluoride (F) intake include drinking water, dental products such as tooth paste, mouth wash, industrial dust and smoke. Ingestion of F along with water converts it to the ionic form which is readily absorbed from the intestinal mucosa. It promptly combines with calcium to form calcium ionospheres that easily permeate the cell membrane. Although fluorides prevent dental caries at low doses, they cause tooth and bone damage at high levels.[3]

Damage caused by fluorosis is not restricted to skeletal tissue and teeth, but also extends to soft tissues, such as brain, liver, kidney, and spinal cord.[4] Excessive F can predominantly inflict DNA destruction which could be attributed to the involvement of oxidative stress. Fluoride can induce production of oxygen free radicals and cause lipid, protein and deoxyribonucleic acid damage. Increased generation of reactive oxygen species (ROS) can readily attack poly unsaturated fatty acids of the cell membrane leading to enhanced lipid peroxidation. F mediated damage of visceral organs could be co-related to the generation of free radicals.[5] Micronuclei (MN) are small round spheric chromatin bodies, appearing in the cytoplasm outside the main nucleus or in the erythrocytes, bearing a close correspondence to the main nucleus of a cell in terms of morphology and staining properties.[6] Formation of MN in bone marrow and peripheral blood could be induced by genotoxic agents like cyclophosphamide, sodium fluoride, cyproterone acetate and heavy metals like chromium and mercury. Micronucleus formation can be induced by free radicals by following exposure to mutagens leading to lipid peroxidation of membrane. The end products of lipid peroxidation could cause breakage of DNA and covalent binding between the product of lipid peroxidation and DNA.[7]

Ocimum sanctum is a widely available medicinal herb found in every household having a wide array of medicinal qualities. *Ocimum sanctum* was found to confront the effect of mutagens. The probability of intercepting genotoxicity induced by F has been undertaken in the current investigation. If a perceptible

response is obtained, it will tremendously benefit natives in this region as *O.sanctum* is indigenous to this area.

Ocimum sanctum (family-Labiatae), commonly known as Sacred basil, is a plant rich in volatile oil comprising of 70% eugenol, methyl eugenol and caryophyllene. Triterpenoids like rosmarinic acid, ursolic acid, alkaloids, saponins, flavonoids, phenylpropane glucosides and tannins exist in this plant.[8, 9] *Ocimum sanctum* has been well documented for its growth promoting, anti-hypertensive, anti-fertility, anti-diabetic, anti-fungal, anti-emetic, anti-spasmodic, cardiac depressant, smooth muscle relaxant, anti-stress, hepatoprotective, anti-cancer, anti-inflammatory, analgesic and radio protective properties.[9, 10, 11]

Ocimum sanctum is capable of protecting DNA from detrimental effects when exposed to xenobiotics like cyclophosphamide, cyproterone acetate[12] and heavy metals like Cr and Hg.[13] However the impact of *Ocimum sanctum* on fluoride induced genotoxicity has not been undertaken so far. The present investigation evaluated the effect of ethyl acetate fraction of *Ocimum sanctum* on fluoride induced micronuclei and oxidative stress in experimental animals.

2. MATERIALS AND METHODS

2.1. Drugs and chemicals

Sodium fluoride (Batch No.D024290179) was purchased from Universal Laboratories Pvt Ltd., Mumbai. May Grunwald-stain and Bovine albumin serum were purchased from Rolex Chemical Industries, Mumbai. Giemsa stain was purchased from Thermo Fischer Scientific India Pvt Ltd., Mumbai. Thiobarbituric acid was purchased from LOBA Chemie Pvt Ltd., Mumbai. All other reagents and chemicals used were of analytical grade.

2.2. Animals

Healthy albino mice weighing 25-30 g were procured from National Institute of Nutrition, Tarnaka, Hyderabad and acclimatized in our animal house for one week prior to the experiment. The animals were maintained at 22 ± 3 ^{0}C under natural light dark conditions and fed on standard diet with *adlibitum* to deflourinated water (RO water). This experimental protocol has been approved by the institutional animal ethical committee (IAEC) and the experiments have been conducted as per guidelines laid down by Committee for the Purpose and Control of Supervision of Experiments on Animals, Chennai, India.

2.3. Plant Material

Leaves of *O. sanctum* were collected from the local areas of Nalgonda district, Telangana, India, and were verified by Professor A. Laxma Reddy, Department of Botanical sciences, Nagarjuna Government Degree College, Nalgonda. A voucher specimen (No. 005) has been deposited in our herbarium.

2.4. Preparation of *Ocimum sanctum* leaf extract

200 g of leaf powder was extracted with 1000 ml of 95 % v/v ethanol in a Soxhlet apparatus at 45-50 °C. Ethanolic extract was subjected to fractionation between hexane and water. The aqueous fraction was collected and partitioned with ethyl acetate. Ethyl acetate fraction (EAF) was collected, concentrated at low temperature and refrigerated. The percentage yield was found to be 0.5% w/w. Preliminary Phytochemical tests were performed to detect the presence of flavonoids.

2.5. Dose selection

Safety evaluation was carried out according to OECD guidelines 425. It was found safe upto 2000 mg/kg. 100, 400 and 800 mg/kg of ethylacetate fraction were selected for the study. The dose of sodium fluoride selected was 30 mg/kg.[14]

2.6. Experimental design

The animals were divided into 7 groups of 6 mice each for the dose dependent and time dependent studies for peripheral and bone marrow micronucleus assay.

2.6.1. Dose dependent study

Group I: Animals were administered with 0.2 ml of 1% w/v acacia by oral route.
Group II: Animals were administered with a single dose of 30 mg/kg of sodium fluoride by the i.p. route to induce micronuclei.
Group III: Animals were treated with 100 mg/kg of EAF, after 1 h they were injected with sodium fluoride (30 mg/kg) by the i.p. route.
Group IV: Animals were treated with 400 mg/kg of EAF, after 1 h they were injected with sodium fluoride (30 mg/kg) by the i.p. route.
Group V: Animals were treated with 800 mg/kg of EAF, after 1 h they were injected with sodium fluoride (30 mg/kg) by the i.p route.

2.6.2. Time dependent study

Group VI: Animals were treated with 100 mg/kg of EAF once daily for 3 days, 1 h after the last dose sodium fluoride (30 mg/kg) was injected i.p.
Group VII: Animals were treated with 100 mg/kg of EAF once daily for 7 days, 1 h after the last dose sodium fluoride (30 mg/kg) was injected i.p.

The ethylacetate fraction was suspended in 1 % w/v acacia and administered to animals by oral route. Blood was withdrawn from the same animals for estimation of oxidative parameters before sacrificing the animals.

2.7. Peripheral blood micronucleus assay

Peripheral blood was collected by retro orbital puncture under enflurane anesthesia in test tubes containing 1% w/v EDTA as an anticoagulant. Smear was prepared on slides, air dried, fixed with absolute methanol for 10 min and stained with phosphate buffer diluted Giemsa (1:4) for 12 min. Slides were observed under microscope for the presence of micronuclei.[15]

2.8. Determination of antioxidant activity by ferric reducing ability of plasma (FRAP) assay

Plasma antioxidant status was evaluated using ferric reducing antioxidant power (FRAP) assay. The assay was carried out according to F.F. Benzie and J.J. Strain (1996). 100 µl of plasma was mixed with 3 ml of working FRAP reagent and absorbance was measured at 0 min after vortexing at 593 nm. Thereafter, samples are placed at 37 °C in water bath and absorbance was again measured after 4 min. Ascorbic acid standards (100 µM-1000 µM) were processed in the same way.[16]

2.9. Estimation of Thiobarbituric acid Reactive Substances (TBARS) in plasma

The thiobarbituric acid reactive substances levels were estimated as per the spectrophotometric method described by Ohkawa et al., 1987. 0.5 ml of plasma was mixed with 0.5 ml of normal saline, 1 ml of 20 % w/v trichloroacetic acid (TCA) and 0.67 % w/v TBA reagent. The samples were kept for boiling at 95 ºC for 1 h. To each of the sample, 3 ml of n-butanol was added, mixed well and centrifuged at 856.8 g for 10 min. The separated butanol layer was collected and read in a spectrophotometer against reagent blank at 535 nm. TBARS concentration was expressed in terms of µmol of malondialdehyde per milliliter of plasma.[17]

2.10. Bone marrow micronucleus assay

The same experimental animals were used for both peripheral blood MN and bone marrow MN assays. Animals in all groups were sacrificed by cervical dislocation 24 h after the intraperitoneal injection of sodium fluoride. Animals were dissected to excise femur. Marrow suspension from femur bone was prepared in 5% bovine serum albumin (BSA) and centrifuged at 100.8 g. The pellet formed was resuspended in a required quantity of BSA. A drop of this suspension was taken on clean glass slides, smears were prepared and the slides were air-dried. The slides were fixed in methanol, stained with May-Gruenwald-Giemsa and MN were identified in two forms of red blood cells which are Polychromatic Erythrocytes as PCE and Normochromatic Erythrocytes as NCE using binocular microscope under oil immersion objective. About 500 PCE and corresponding NCE per animal were observed for the presence of MN.[15]

2.11. Statistical analysis

Data are expressed as mean ± SEM. Analysis of data was done by One-way ANOVA followed by Dunnett comparison test for FRAP and TBARS, Mann-Whitney U test was carried out for micronuclei in bone marrow and peripheral blood using Graph Pad In Stat version 3.10 for Windows 2009 (Graph Pad Software). The statistical significance was set as 0.01 and 0.05 ($P<0.01$, $P<0.05$).

3. RESULTS

3.1. Peripheral blood micronucleus assay

Formation of MN (MNNCE) was evident within 24 h of a single intraperitoneal exposure with sodium fluoride (30 mg/kg). Treatment with 800 mg/kg of EAF exerted a significant reduction in the frequency of formation of MNNCE induced by F indicating that high dose of EAF is more effective in prevention of MN compared with the sodium fluoride treated group ($P<0.01$) The number of MNNCE observed with 100 and 400 mg/kg of EAF also declined (Table I). In time course studies, treatment with a dose of 100 mg/kg of EAF for 7 days exerted a significant reduction in the formation of MNNCE compared with the sodium fluoride treated group ($P< 0.01$, Table I).

3.2. Determination of antioxidant activity by ferric reducing ability of plasma (FRAP) assay

A dose dependent increase in antioxidant activity was observed, however treatment with 800 mg/kg of EAF was most efficacious in terms of antioxidant capacity (700 µmol/L) compared to 100 mg/kg and 400 mg/kg of EAF. 100 mg/kg administered for 7 days was found to possess greater antioxidant capacity (600 µmol/L) which was significant compared with the sodium fluoride treated group ($P<0.01$, Fig. 1).

3.3. Estimation of Thiobarbituric acid Reactive Substances (TBARS) in plasma

Oxidative stress represented by the concentration of TBARS was found to be less in group-3 treated with 800 mg/kg than animals treated with 100 mg/kg and 400 mg/kg of EAF. When treatment was continued for 3 and 7 days with 100 mg/kg, a significant reduction in the formation of oxidative stress markers was observed ($P< 0.01$, Fig. 2).

3.4. Bone marrow micronucleus assay

In dose-dependent study, the number of MNPCE and MNNCE were reduced in groups treated with 100 mg/kg and 400 mg/kg of EAF. The group treated with 800 mg/kg of EAF exhibited a clear reduction in MNPCE and MNNCE formed compared with the sodium fluoride treated group ($P<0.01$). In the time dependent studies, the proportion of MNPCE and MNNCE formed declined as the duration of treatment with EAF (100 mg/kg) progressed ($P< 0.01$, Table I).

Table I: Effect of ethylacetate extract of *Ocimum sanctum* on the frequency of micronuclei in bone marrow and peripheral blood induced by sodium fluoride.

Treatment	Bone marrow micronucleus test	Peripheral blood	micronucleus test
	% MNPCEa	% MNNCEb	% MNNCEc
Negative control	0.4±0.12	0.2267±0.08	0 ±0.00
SF (30 mg/kg)	9.2±0.42	0.993±0.09	3 ±0.26
ETOAC (100 mg/kg)	1.4±0.05**	0.2698±0.07**	0.233±0.06**
ETOAC (400 mg/kg)	1.83±0.21**	0.3675±0.10**	0.266±0.04**
ETOAC (800 mg/kg)	3.4±0.36**	0.5617±0.14*	0.33±0.04**
ETOAC (100 mg/kg) for 3 days	0.933±0.12**	0.142±0.05**	0.133±0.04**
ETOAC (100 mg/kg) for 7 days	0.4±0.15**	0.000±0.0	0.066±0.04**

Values are expressed as Mean ± SEM, n=6, Statistics: Mann-Whitney U test, ** P<0.01,* P<0.05 compared with positive control, a) from 3000 PCE and b) corresponding NCE counted. c) from 3000 NCE. MNPCE: micronucleated polychromatic erythrocytes, MNNCE: Micro nucleated normochromatic erythrocytes, SF: Sodium fluoride, . ETOAC: ethylacetate extract of *Ocimum sanctum*

Determination antioxidant activity by Ferric Reducing Ability of Plasma (FRAP) assay.

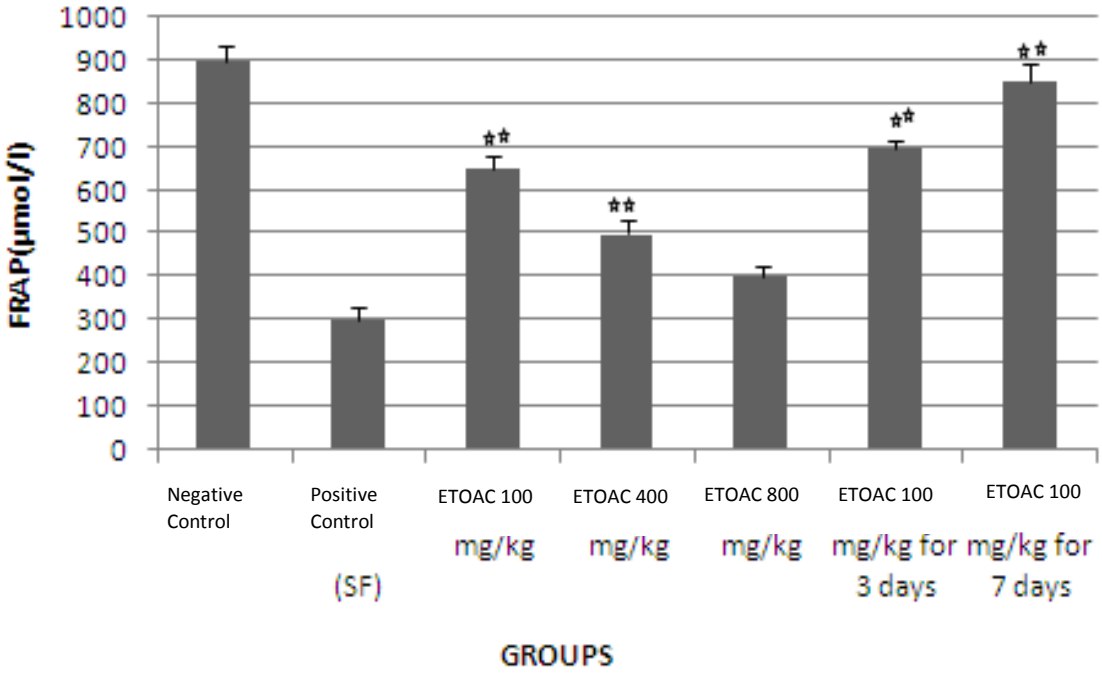


Fig 1: Effect of ethylacetate extract of *O .sanctum* on plasma antioxidant capacity in fluoride induced genotoxicity. Values are Mean ± SEM, n=6, **P<0.01 compared with positive control. ETOAC: ethylacetate extract of *Ocimum sanctum*, SF: sodium fluoride.

Estimation of Thiobarbituric Reactive Substances (TBARS) in plasma.

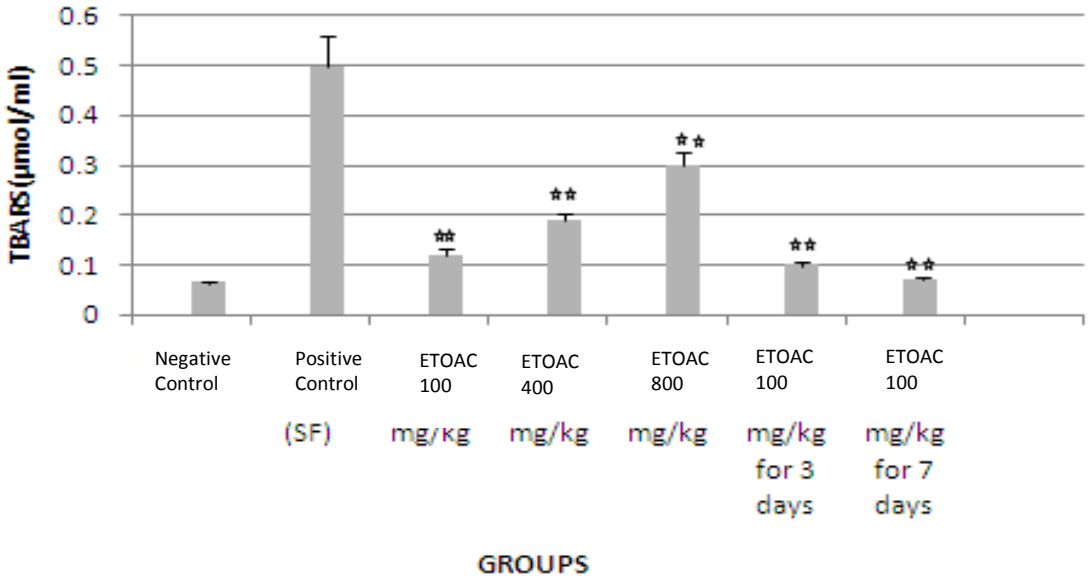
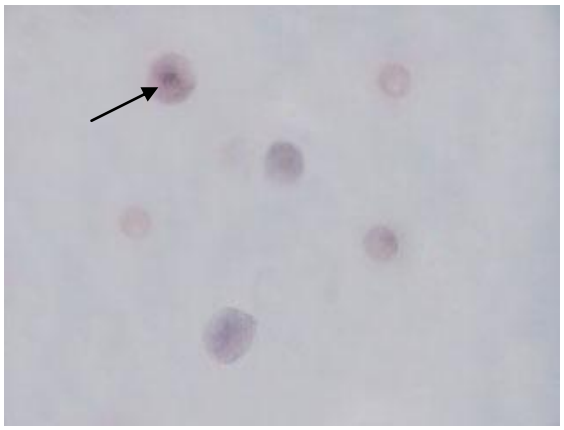
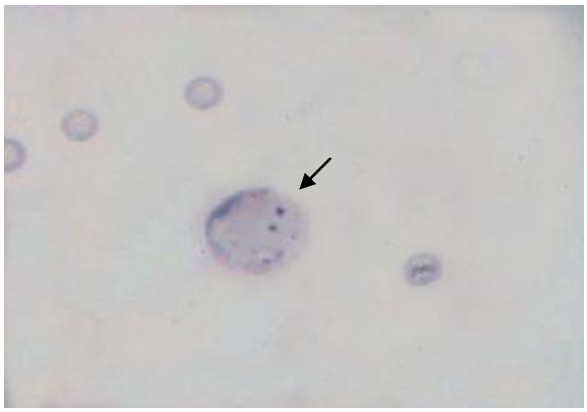


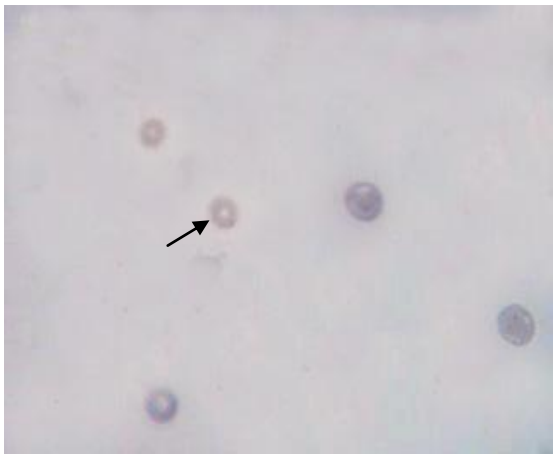
Fig 2: Effect of ethylacetate extract of *O .sanctum* on concentration of TBARS in fluoride induced genotoxicity. Values are expressed as Mean ± SEM, n=6, **P<0.01 compared with positive control. ETOAC: ethylacetate extract of *Ocimum sanctum*, SF: sodium fluoride.



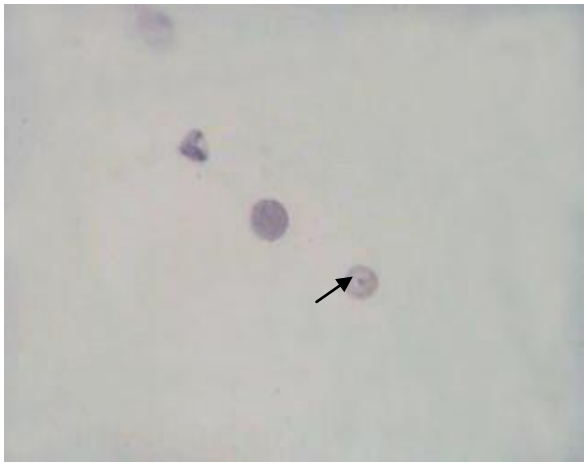
A



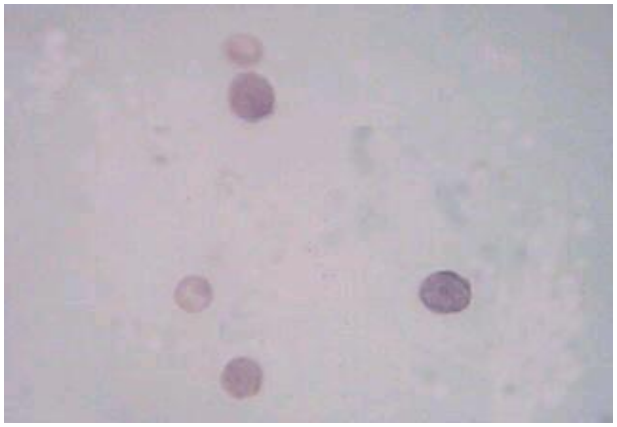
C



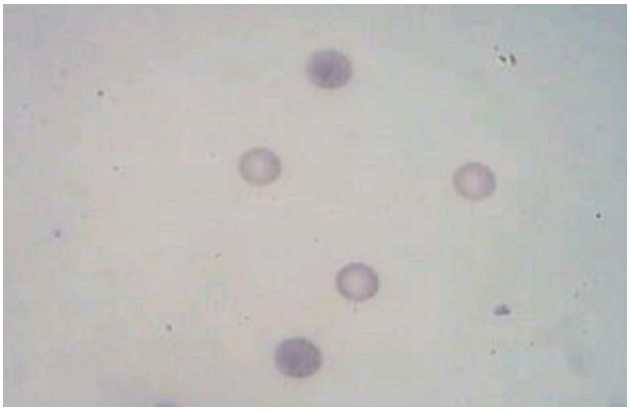
B



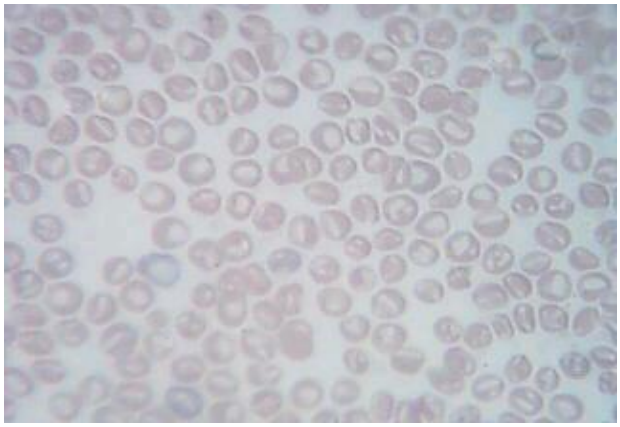
D



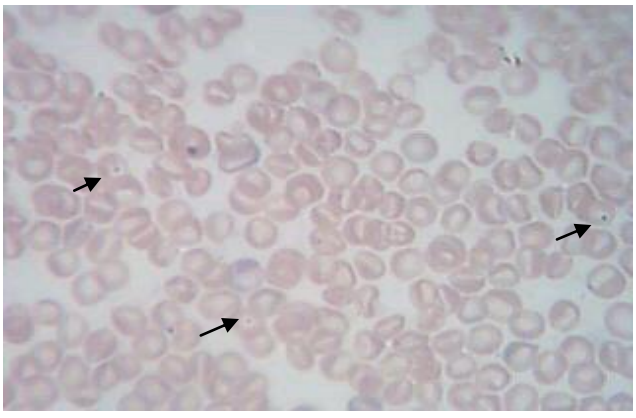
E



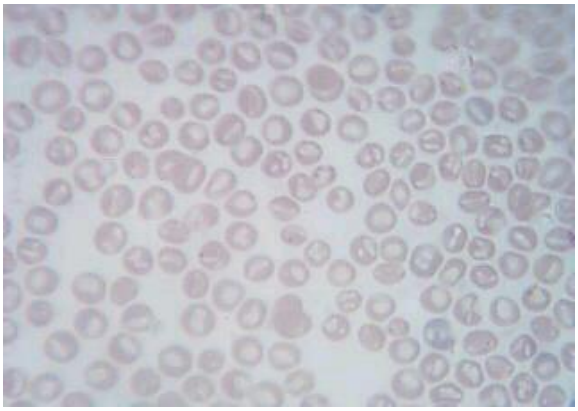
F



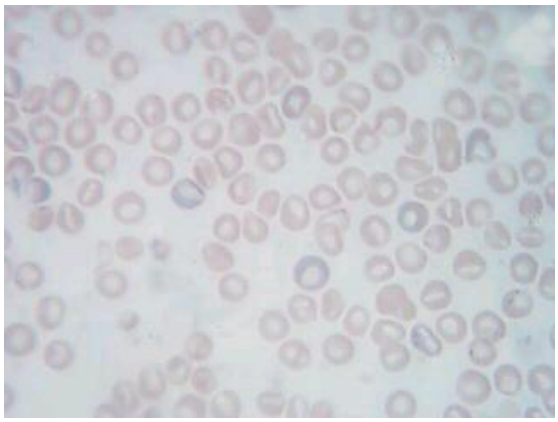
H



I



J



K

Fig 3: Photographs showing micronucleated erythrocytes. A&B shows control photographs of PCE and NCE respectively in bone marrow. C&D shows MNPCE and MNNCE in bone marrow in sodium fluoride treated group. E & F shows ETOAC treatment 100 mg/kg and ETOAC 100 mg/kg for 3 and 7 days respectively in bone marrow. H, I, J, K, represents control, sodium fluoride induced, ETOAC 100 mg/kg, ETOAC 100 mg/kg for 3 and 7 days respectively in peripheral blood.

4. DISCUSSION

The present study was designed to evaluate the protective role of the ethyl acetate fraction of *Ocimum sanctum* on micronuclei induced by fluoride. F has a propensity to cumulate in the body as only 50 % is cleared by the kidneys. The remaining fluoride tends to accumulate in bones, pineal gland and other tissues. By the process of simple diffusion, F can readily penetrate the cell membrane[2] and damage DNA and RNA. F induces damage to nucleic acids by multiple mechanisms which include interaction with calcium and magnesium which affect the working of DNA polymerase and RNA enzymes thus interfering with the formation of DNA, RNA and proteins. F readily binds to NH residues and interferes with adenine-thymine base pairing causing alteration in DNA replication. F is capable of interfering with the functioning of several enzymes and affects H bonding.[18] F can induce oxygen metabolism and facilitate the production of oxygen free radicals. There is much evidence supporting the claim that free radicals are

important mediators of fluoride-induced toxicity. NaF can significantly increase formation of lipid peroxides in kidney and liver, as evidenced by the enhanced TBARS levels.[5] Apart from DNA damage, Anuradha *et al*[19] have reported that sodium fluoride (NaF) is capable of triggering programmed cell death due to oxidative stress. This can induce the liberation of cytochrome C into the cytosol, which can further instigate cellular apoptosis. F can produce deleterious effects on the DNA due to which it has consolidated its position as a genotoxicant and has been employed in our study.

Cell division involves the segregation of chromosomes equivocally to two daughter cells. In some instances, unrepaired chromosomal damage can result in the formation of MN which might not be included in the main daughter nuclei during cell division. Exposure to mutagens, defects in cell cycle checkpoints and DNA repair genes might be affected thereby increasing the chance of MN formation. Micronucleus formation could ensue due to free radical generation from any agent that facilitates lipid peroxidation of membrane leading to breakage of DNA.[20] The bone marrow micronuclei assay is a popular cytogenic assay developed by Schmid to detect the appearance of MN.[21]

Our studies reveal significant elevation in the formation of PCE and NCE in both the bone marrow and peripheral blood following challenge with sodium fluoride. The frequency of micronuclei formation significantly diminished with EAOS both in bone marrow and peripheral blood with elevated doses. A similar response was also observed as the duration of treatment progressed.

Oxidative stress is often assessed by the levels of lipid peroxides generated in the inflicting process. TBARS assay is sensitive and reliable as it is capable of measuring lipid peroxidation products such as malondialdehyde, alkanals, alkenals, hydroalkenals as well as ketones.[22] A significant elevation in the formation of TBARS was observed in the sodium fluoride exposed group compared with the control. Treatment with ETOAC exhibited a dose dependent reduction in the formation of lipid peroxides. The FRAP assay involves the reduction of ferric to ferrous ions which can be catalyzed by antioxidants. Oxidative damage produced by free radicals could be circumvented by antioxidants due to their reducing ability. Antioxidants behave as efficient reducing agents as they are generous in donating an electron or a hydrogen atom.[23] The FRAP values were the highest with 800 mg/kg of ETOAC and exerted a similar effect as the duration of treatment increased.

Leaves of *Ocimum sanctum* are rich in essential oils. The presence of eugenol in considerable amounts could be responsible for controlling lipid peroxidation as report by Sethi et al.,[24] suggest that eugenol possesses significant antioxidant property. Preliminary phytochemical tests have confirmed the presence of flavonoids in the ethyl acetate fraction. Flavonoids are plant derived polyphenols possessing a constellation of biological effects like anti-inflammatory, anti-allergic, anti-viral, anti-cancer and radio protective effects in vitro and in vivo. Flavonoids and tannins have a distinct ability of chelation in addition to their free radical scavenging properties which can contribute to their antioxidant activity.[6, 25] Being polyphenols, flavonoids are excellent scavengers of free radicals due to the high reactivity of their hydroxyl substituents. Mitsuo miyazawa et al.,[26] have documented the anti-mutagenic activity of flavonoids like apigenin, luteolin and quercetin. Isoquercetin, kaempferol, apigenin, luteolin, rosamarinic acid and oleanolic acid act as antioxidants and could be present in the ethylacetate fraction.[27]

It has been proposed that polyphenols could exert their antimutagenic effect by enhancing the functioning of DNA repair enzymes by modulating their gene expression[28] or by adsorbing the mutagen thereby impeding mutagenesis[29] or by inhibiting the formation of toxic metabolites and reducing the formation of DNA adducts.[30] Genomic stability was altered following challenge with NaF which was significantly averted with the ethyl acetate fraction of *Ocimum sanctum.* This implies that reduction of micronuclei formation by *Ocimum sanctum* could be attributed to any of these mechanisms.

In conclusion, ethyl acetate fraction of *Ocimum sanctum* was capable of protecting DNA, a sensitive intracellular target from conspicuous damage by sodium fluoride and could be recommended as a natural repressor of fluoride induced toxicity.

REFERENCES

1. A.S. Narayana, A.L. Khandare, M.V.R.S Krishnamurthi, Mitigation of Fluorosis in Nalgonda District Villages, 4th International. Workshop on Fluorosis Prevention and Defluoridation of Water, ISFR, EnDeCo, ICOH 98-106.
2. S. Chouhan, S.J.S. Flora, Effects of fluoride on the tissue oxidative stress and apoptosis in rats:Biochemical assays supported by IR spectroscopy data, Toxicol, 2008; 254: 61–67.
3. M. Kanbur, G. Eraslan, S. Silici, M. Karabacak, Effects of sodium fluoride exposure on some biochemical parameters in mice: Evaluation of the ameliorative effect of royal jelly applications on these parameters, Food and Chemical Toxicology, 2009; 47: 1184–1189.
4. M. Zhanga, A. Wanga, T. Xiaa, P. Hea, Effects of fluoride on DNA damage, S-phase cell-cycle arrest and the expression of NF-_B in primary cultured rat hippocampal neurons, Toxicology Letters, 2008; 179: 1–5.
5. H. Bouaziz, F. Croute, T. Boudawara, J. P. Soleilhavoup, N. Zeghal, Oxidative stress induced by fluoride in adult mice and their suckling pups,

Experimental and Toxicologic Pathology, 2007; 58: 339–349.
6. J.Ganesh Chandra, T. K. Reddy, The grapefruit flavanone naringin protects against the radiation-induced genomic instability in the mice bone marrow: a micronucleus study, *Mutat Res,* 2002; 519: 37–48.
7. O. Awodele, S.O. Olayemi, C.G. Alimba, C. Egbejiogu, A. Akintonwa, Protective effect of vitamin C and or vitamin E on micronuclei induction by rifampicin in mice, Tanzania Journal of Health Research, 2010; 12: 1-7.
8. Geetha, R. Kedlaya, D.M. Vasudevan, Inhibition of lipid peroxidation by botanical extracts of *Ocimum sanctum*: In vivo and in vitro studies, Life Sciences, 2004; 76: 21–28.
9. M.R.Adhvaryu, S.P. Srivastav, S.N.Vaniawala, M.N. Reddy, A comparative study of radioprotection by four Indian medicinal herbs against genotoxicity induced by sub-lethal gamma irradiation in Swiss albino mice, Iran. J. Radiat. Res, 2008; 6: 19- 30.
10. S. Shetty, U. Saraswati, U. Laxminarayana, Evaluation of antioxidant and wound healing Effects of alcoholic and aqueous extract of *Ocimum sanctum* Linn in Rats, eCAM, 2007; 1- 7.
11. P. Prakash, N. Gupta, Therapeutic uses of *ocimum sanctum linn* (tulsi) with a note on eugenol and its pharmacological actions: a short review, Indian J Physiol Pharmacol, 2005; 49: 125–131.
12. Y. H. Siddique, A. Gulshan, T. Beg, M. Afzal, Anti-genotoxic effect of *ocimum sanctum* L. extract against cyproterone acetate induced genotoxic damage in cultured mammalian cells, Acta Biologica Hungarica, 2007; 58: 397–409.
13. K. Babu, K.C. Uma Maheswari, In vivo studies on the effect of *Ocimum sanctum* L. leaf extract in modifying the genotoxicity induced by chromium and mercury in Allium root meristems, Journal of Environmental Biology, 2006; 27: 93-95.
14. Y. Suzuki, J. Li, H. Shimizu, Induction of micronuclei by sodium fluoride, *Mutat Res*, 1991; 253-278.
15. K.P. Seetha Rama Rao, K.Narayana, Invivo chromosome damaging effects of an inosine monophosphate dehydrogenase inhibitor: Ribavirin in mice, Indian J Pharmacol, 2005; 37: 90–95.
16. FF. Benzie, JJ. Strain, The ferric reducing ability of plasma (FRAP) as a measure of ‘‘Antioxidant Power’’: The FRAP Assay, *Anal Biochem*, 1996; 236: 70–76.
17. R. Premanand, P.H. Santhosh Kumar, A.Mohan. Study of thiobarbituric reactive Substances and total reduced glutathione as indices of oxidative stress in chronic smokers with and without chronic obstructive pulmonary disease, *Indian J Chest Dis Allied Sci*, 2007; 49: 9–12.
18. Xiu An Zhan, Min Wang_, Zi Rong Xu, Wei Fen Li, Jian Xin Li, Effects of fluoride on hepatic antioxidant system and transcription of Cu/Zn SOD gene in young pigs, Journal of Trace Elements in Medicine and Biology, 2006; 20: 83–87.
19. CD Anuradha**,** S Kanno, S Hirano, Oxidative damage to mitochondria is a preliminary step to caspase-3 activation in fluoride-induced apoptosis in HL-60 cells, *Free Radic Biol Med*, 2001; 31: 367-373.
20. B. Stefano, Z. Ariana, C. Marcello, L. Cecilia, P.C. Wushou, H.Nina, K.V. Micheline, Z. Errol, B. Sadayuki, B. Roberto, P.B.Maria, B. Claudia, C.W. Antonina, F. Eleonora, F. Alexandra, H. Lars, J. Gordana, M. Antonietta, M. Lucia, M. Ekaterina, R.S. Maria, Z. Andrea, N. Hannu, F. Michael, An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans, Carcinogenesis, 2007; 28: 625–631.
21. Syed Imam Rabbani, Kshama Devi, Salma Khanam, Noor Zahra, Citral, a component of lemongrass oil inhibits the clastogenic effect of nickel chloride in mouse micronucleus test system, Pak. J. Pharm. Sci, 2006; 19: 108-113.
22. C.M. Seppanen, A. Saari Csallany, Hydroxynonenal, a Toxic aldehyde, in Soybean Oil at Frying Temperature, JAOCS, 2002; 79: 10.
23. A. Lamien-Meda, C.E. Lamien, M.M. Compaoré, R.N. Meda, M. Kiendrebeogo, B. Zeba, J.F. Millogo, O.G. Nacoulma, Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso, Molecules, 2008; 13: 581-594.
24. Jyoti Sethi, Sushma Sood, Shashi Seth, Anjana Talwar, Evaluation of hypoglycemic and antioxidant effect of *Ocimum sanctum*, Indian Journal of Clinical Biochemistry, 2004; 19: 152-155.
25. K.E. Heim, A.R. Tagliaferro, D.J. Bobilya, Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships, Journal of Nutritional Biochemistry, 2002; 13: 572–584.
26. Mitsuo Miyazawa, Masayoshi Hisama, Anti-mutagenic activity of flavonoids from Chrysanthemum morifolium, Biosci. Biotechnol. Biochem, 67: 2091-2099.
27. A. Gangwal, S.K. Parmar, N.R. Sheth, Triterpenoid, flavonoids and sterols from *Lagenaria siceraria* fruits, Der Pharmacia Lettre, 2010; 2: 307-317.
28. T. Ohta, Modification of genotoxicity by naturally occurring flavorings and their derivatives, Crit. Rev. Toxicol, 1993; 23: 127–146.
29. L.R. Ferguson, P.J. Harris, Suberized plant cell walls suppress formation of heterocyclic amine-induced aberrant crypts in a rat model, Chem.Biol. Interactions, 1998; 114: 191–209.
30. W.A. Smith, J.M. Arif, R.C. Gupta, Effect of cancer chemopreventive agents on microsome-mediated DNA adduction of the breast carcinogen dibenzo[a,l]pyrene, Mutat. Res, 1988; 412: 307–314.