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Original Article

Cigarette smoke induced oxidative insult in local population of Pokhara

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Abstract

Objectives: To assess the effect of cigarette smoking on lipid peroxidation induced oxidative stress, antioxidants, uric acid and blood sugar in normal subjects.

Methods: The study included 61 normal subjects with regular smoking habit and 57 never-smokers normal subjects matched in respect to socio-economic status, age and BMI. Information regarding smoking habit and other personal details were collected by oral questionnaire. Total antioxidant activity (TAA), reduced glutathione (GSH), α-tocopherol (α-T**),** ascorbic acid (AA), uric acid (UA), plasma and urinary thiobarbituric acid reactive substances (TBARS), fasting blood sugar (FBS) and urinary creatinine (Cr) were estimated by standard procedures in both the groups. Ferric Reducing Antioxidant Power (FRAP) procedure is used to estimate TAA which measures total dietary antioxidants. Statistical analysis was done with SPSS version 10.

Results: The mean pack years smoked by smokers was 14.4 ± 15.8. The plasma TBARS level in smokers and never-smokers was 2.6 ± 0.8 and 2.5 ± 0.6 μmol/L respectively. The respective figure for urinary TBARS level was 4.6 ± 2.7 and 3.7 ± 1.4 μmol/gmCr. Smokers did not show any significant difference from never-smokers with respect to GSH, α-T, AA, plasma TBARS and FBS. However, the smokers had significantly lower levels of TAA ($p<0.05$) and raised level of urinary TBARS ($p<0.05$) and uric acid ($p<0.01$) as compared to never-smokers.

Conclusion: Our study suggests that smoking induces mild lipid peroxidation but the body is able to compensate for it by removing its adducts. Importantly it also indicates enhanced oxidation of purines which are essential components of both DNA and RNA. Dietary antioxidants are consumed to scavenge free radicals (FR) and other reactive species (RS) in smoke. Female smokers are more prone to oxidative insult than male smokers. In summary RS present in smoke induce mild lipid peroxidation but are not the major contributors of redox imbalance in smoke induced toxicity in the selected subjects.

Key words: Tobacco, Smoking, Free radicals, Oxidative stress, Antioxidants

Smoking is today provenly recognized as lethally toxic to human system as each cigarette tears away 7-11 minutes of human life[1]**.** Presently, about 1 billion males and 250 million females smokes cigarette / bidi and about 5 million people die every year from tobacco induced toxicity and unless drastically effective measures are taken the figure is expected to double by 2025. The scenario is still grimmer in developing and deprived populations where deaths are expected to rise soon to 7 million. Dr. Harlem, Director General, WHO (2002) in her message ruefully warned, "One person dies every 10 seconds due to smoke related disease. Tobacco is a killer. It shouldn't be advertised, subsidized or glamorized"[1]. Besides numerous other diseases and related complications, smoking is a member of dangerous group of risk factors in cancer, cardiovascular diseases, diabetic complications and lung diseases and its assault increases in the state of poor nutrition[2]. Ironically, the smoking is highly prevalent in Nepal and WHO has estimated it to be 38.5% of total population[1] but the figure is based on a small survey and is decidedly underestimated. Pandey et al[3] reported it to be 73.7%. Another study from rural Nepal reported that sulpha is the most common form of smoking in this population, wherein tobacco is directly smoked from an earthen container and that 85% males and 72% females smoked it[4]. This type of smoking is still more harmful as crude tobacco is smoked directly and its smoke mimics to bidi smoke.

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In view of extreme hazards, the causes of tobacco toxicity have been under intense and elaborate examination at molecular level in different cohorts to firmly establish the etiopathogenesis so that discrete protocol for taking up preventive and treatment measures could be planned. However, similar studies are not traceable on Nepali population. The smoke of processed cigarette contains nearly 4700 chemicals, majority of which have multiple harmful effects[5]. Incidentally, many of these chemicals are free radicals or free radicals derived radical species which will be collectively referred here in as reactive species (RS). Further, many of the other species of smoke get transformed to RS in metabolic system *in vivo* in human body. Since, RS by nature are highly aggressive molecules with confirmed diversified capability to distort and destroy cellular structures and its molecular network[6,7] and that cigarette smoke is overcharged with these species, several workers postulated them to be important co-participants in smoke toxicity with the demonstration that oxidative stress (OS) is raised in smokers and is often accompanied by weak antioxidant defense[8,9,10]. Nonetheless, these claims are neither universally applicable nor correlate with the cigarette smoke induced damage proportional with the freight of free radicals present in it[11,12,13,14,15]. Moreover, why different cohorts with comparable nutritional status and smoking behavior show variation with respect to OS and antioxidant defense status is another poorly understood phenomenon.

Although the possible influence of free radical impact and antioxidant defense system has been examined in Cardiovascular disease (CVD) and cancer in Nepali population[12,13,14], to the best of our knowledge no study has been carried out to examine smoke induced oxidative insult in them. In the present report these aspects have been explored by measuring oxidative stress and selected antioxidants in smokers and comparable never- smokers.

Materials and methods

This study was carried out in the Department of Biochemistry, Manipal Teaching Hospital, Pokhara, Nepal. The study included 61 healthy smokers (males= 47, females= 14) and 57 never-smokers (males=31, females= 26) from the local population of Pokhara and were matched with respect to age and socioeconomic status. Height and weight of all subjects was taken and Body Mass Index (BMI) was calculated from the standard chart. All smokers used 85 mm filter cigarettes. Verbal consent was obtained from all the subjects prior to their inclusion in the study. The desired information from both smokers and never-smokers was collected on a pre-tested proforma. Our study was designed to know the cigarette smoke induced oxidative insult in the local population. We, therefore, used biochemical parameters plasma TBARS as a marker of oxidative stress and TAA, GSH, α-tocopherol**,** ascorbic acid, uric acid as a marker of antioxidant status of the body. In addition, we included FBS to know the effect of the smoking in the glycemic status.

Six ml of fasting blood sample was collected from each participant by standard venipuncture technique and the sample was dispensed into EDTA (sodium salt) containing vials. After gentle mixing, 1 ml of whole blood was used for the determination of reduced glutathione (GSH)[16] and hemoglobin (Hb). The remaining sample was centrifuged at 3000 rpm for 10 minutes and plasma was separated. The total antioxidant activity (TAA)[17], fasting blood sugar (FBS) and uric acid (kit Ranbaxy) were measured immediately. Rest of the sample was stored in deep freeze and was analyzed for thiobarbituric acid reactive substances (TBARS)[18], α-tocopherol[19] and ascorbic acid[20]. The spot urine sample was also collected and urinary TBARS and creatinine (kit Ranbaxy) were measured and expressed in term of TBARS and creatinine ratio to mitigate the diurnal variation of TBARS excretion.

Statistical analysis

The results are reported as mean ± SD. The statistical analysis was done with SPSS 10 version software. Independent sample't' test and Pearson's correlation coefficients were calculated wherever applicable and for all determinants, $p<0.05$ was considered statistically significant.

Results

Age, BMI, plasma and urinary TBARS, TAA and individual antioxidants like α- tocopherol, ascorbic acid, uric acid and GSH levels in smokers and never-smokers are given in Table 1. Age and BMI were comparable between smokers and never-smokers. The average pack years smoked was 14.4±15.8. The plasma TBARS levels in smokers and never-smokers did not show any significant difference, but its excretion in urine was significantly higher in the former ($p<0.05$). The smokers had significantly lower level of TAA as compared to non-smokers ($p<0.05$). The α- tocopherol, ascorbic acid and GSH levels did not show any significant difference. The plasma uric acid level was also significantly raised in smokers ($p<0.01$).

Table 2 shows the sex-wise differences in both smokers and never-smokers and some very interesting observations are discernible. A

comparison between male smokers vs male never-smokers shows that smoking has no influence on oxidative stress or on individual antioxidants but TAA is significantly decreased (<0.05). However, smoking shows some different trends in the females. In them, smoking raises OS and uric acid and decreases GSH and TAA. A sex-wise verification reveals that male smokers have significantly raised TAA and uric acid as compared to female smokers with no difference in other parameters. Among never-smokers males have lower GSH and raised TAA and uric acid. The data taken together suggests: a) smoking might not always cause oxidative stress; b) smoking definitely reduces TAA, suggesting that there is more generation of free radical due to smoking and the consumption of dietary antioxidant is significantly increased to compensate this process; c) smoking tends to significantly increase uric acid level. The rise in uric acid indicates that smoking increases oxidation of purines and in turn oxidation of purine is accompanied by additional production of superoxide anion since xanthine oxidase is the potent producer of superoxide anion. This means that smoking aggravates nucleic acids oxidation of which a possible consequence will be damage to nucleic acid specially DNA which is more prone to oxidation as compared to RNA by free radicals.

The relationship between different parameters is given in Table 3. These statistical analyses relay useful information. Some of the important features requiring attention are: a) uric acid significantly contributes to TAA in smokers ($p<0.01$) but doesn't follow a significant relationship with plasma TBARS inferring that other antioxidants are equally important to participate in reducing the oxidative stress; b) the most important feature is a positive relationship between PTBARS vs UTBARS in smokers. This shows that within physiological limits body tends to metabolize lipid peroxidation adducts which are proportionately excreted out by the kidney in urine; c) in never-smokers, uric acid showed significant relationship both with plasma TAA and TBARS. A negative correlation was noted between plasma TBARS vs GSH in smokers. Which suggests that reduced glutathione is proportionately consumed by RS in smoke. Since no significant difference was noted in GSH levels between smokers and never-smokers, it can be interpreted to mean that GSH consumed is proportionately regenerated also. Strikingly, the plasma TBARS vs plasma TAA relationship was positive, suggesting a consistent use of TAA for scavenging RS. The analysis of the data on the basis of duration of smoking and pack year smoked did not show any significant conclusive trend.

Table 1: Lipid peroxidation and antioxidants levels in never- smokers and smokers

Parameters	Never-smokers (n=57) Mean ± SD	Smokers (n=61) Mean ± SD
Age (years)	36.5 ± 17.6	38.3 ± 15.1
BMI (kg/m^2)	22.7 ± 3.5	21.8 ± 3.0
Pack years	NIL	14.4 ± 15.8
GSH (mg/gmHb)	2.8 ± 0.7	2.7± 0.7
GSH (mg/dl whole blood)	37.0 ± 8.5	37.4 ± 9.9
TAA (µmol/L)	664 ± 192	585 ± 201[a]
α-tocopherol (mg/dl)	0.90 ± 0.32	0.86 ± 0.40
Ascorbic acid (mg/dl)	0.78 ± 0.25	0.75 ± 0.23
Uric acid (mg/dl)	4.7 ± 1.4	5.7 ± 1.6[b]
Fasting blood sugar (mg/dl)	81 ± 10	84 ± 10
Plasma TBARS (µmol/L)	2.5 ± 0.6	2.6 ± 0.8
Urine TBARS (µmol/gmCr)	3.7 ± 1.4	4.6 ± 2.7[a]

'p' value: a= <0.05, b= <0.01, c= <0.00

Table 2: Lipid peroxidation and antioxidants levels in male and female never-smokers and smokers

Parameters	Never-smokers		Smokers	
	Male (n=31) Mean ± SD	Female (n=26) Mean ± SD	Male (n=47) Mean ± SD	Female (n=14) Mean ± SD
Age (years)	37.9 ± 16.4	34.8 ± 19.3	39.0 ± 16.7	36.1 ± 7.8
BMI (kg/m^2)	23.5 ± 3.4	21.8 ± 3.6	22.5 ± 3.0	19.8 ± 2.0[b]
Pack years	Nil	Nil	14.5± 16.2	14.4± 14.9
GSH (mg/gmHb)	2.5 ± 0.6	3.2 ± 0.7[c]	2.7 ± 0.8	2.9 ± 0.5[1]
GSH (mg/dl whole blood)	35.3 ± 9.2	39.0 ± 7.3	38.5 ± 11.0	33.6 ± 5.4
TAA (µmol/L)	742 ± 198	570 ± 135[c]	628 ± 190[1]	442 ± 172[b, 1]
α-tocopherol (mg/dl)	0.94 ± 0.32	0.86 ± 0.32	0.87 ± 0.33	0.80 ± 0.59
Ascorbic acid (mg/dl)	0.76 ± 0.22	0.81 ± 0.30	0.76 ± 0.22	0.72 ± 0.27
Uric acid (mg/dl)	5.5 ± 1.5	3.9 ± 0.8[c]	6.1 ± 1.5	4.6 ± 1.4[b]
Fasting blood sugar (mg/dl)	82 ± 11	80 ± 8.4	84 ± 10	83 ± 11
Plasma TBARS (µmol/L)	2.6 ± 0.6	2.3 ± 0.7	2.7 ± 0.8	2.5 ± 0.9
Urine TBARS (µmol/gmCr)	3.5 ± 1.5	4.0 ±1.3	4.5 ± 2.9	5.2 ± 1.9[1]

'p' value: when male never-smokers and male smokers are compared with female never-smokers and female smokers: a= <0.05, b= <0.01, c= <0.001

'p' value: when male and female never-smokers are compared to their smoker counterparts: 1= <0.05, 2= <0.01, 3= <0.00

Table 3: Pearson's correlation coefficients

Parameters	Pearson's correlation coefficients (r)	p value
Plasma TAA Vs Uric acid in smokers	0.413	<0.01
PTBARS Vs Uric acid in smokers	0.105	NS
Plasma TAA Vs Uric acid in never-smokers	0.480	<0.01
PTBARS Vs Uric acid in never-smokers	0.301	<0.05
PTBARS Vs UTBARS in smokers	0.270	<0.05
GSH Vs PTBARS in smokers	-0.260	<0.05
GSH Vs Uric acid in smokers	-0.302	<0.05
FBS Vs PTBARS in smokers	0.078	NS
FBS Vs PTBARS in never-smokers	-0.083	NS

PTBARS= Plasma TBARS, UTBARS= Urine TBARS, NS=Not Significant

Discussion

In the present study, the average number of cigarette smoked per day was 12.1 ± 7.5 sticks for a period of 18.7 ± 13.8 years. If the report of Pryor and Stone[21] is taken as correct figure then each cigarette generates 15,000 trillion molecules of RS and still more are induced in vivo. They were thus directly exposed to a shocking number of about 181,500 trillion molecules of RS every day for prolonged period. Among 61 smokers 25 persons smoked ≥ 20 cigarettes per day (22.2 ± 4.6 cigarette/day) over a period of 27.4 ± 14.0 years. They were directly exposed to 333,000 trillion molecules of RS every day. The inflicting reactivity of these aggressive RS and their adducts is well documented in literature[22]. The exposure of aforesaid quantity of RS in these smokers is, therefore, logically supposed to induce extensive tissue and metabolic damage accompanied with RS associated diseases, but none of the subjects included in this study showed any overt symptoms of acute or chronic illness. These observations, hence, do not sustain the hypothesis that RS in smoke are primary culprits in its toxicity and there may be other environmental factors and genetic factors associated with it.

The above deduction is further supported by our biochemical findings. The lipid peroxidation induced OS was measured in terms of plasma and urinary TBARS levels. The plasma TBARS levels did not show any significant difference between smokers (2.6 ± 0.8 μmol/L) and never-smokers (2.5 ± 0.6 μmol/L). However, smokers excreted them significantly more (about 25%) than the never-smokers. This infers that though the OS in smokers is not raised, the lipid peroxidation is increased. Thus, the body is able to meet this challenge through its compensatory mechanisms and one of them being excretion of peroxidative adducts through the kidneys **[23]**. Siu and Draper **[23]** carried out extensive in vivo studies to examine the metabolism and excretion of MDA, a surrogate marker of lipid peroxidation and demonstrated that 9-17% of it is excreted in urine.

Our assumption is also supported by Merken et al[24] who noted normal MDA levels both in normal subjects and COPD patients but the latter group had its raised urinary excretion. Risal et al[13] did not find any difference in plasma TBARS levels between smokers and never-smokers. Contrary to our observations, several workers have noted raised OS in smokers[9,25,26,27]. Jain et al[25] examined the TBARS levels in bidi smokers and noted that the TBARS levels were about two times and four times more in mild smokers and heavy smokers respectively. Notably, Lykkesfeldt et al[26] observed high plasma MDA levels in smokers, inspite of balanced antioxidant status. Yang et al[27] noted stimulated secretion of pro-inflammatory cytokines in lungs in smokers and attributed it to smoke induced OS. There is still another group which has noted lower OS in smokers[11,12]. Recently, in an excellent study Patel et al[28] noted lower OS in oral cancer patients due to tobacco chewing and attributed it to geared antioxidant defenses.

The data on antioxidant status in smokers are still more variable and conflicting. Among antioxidant defense system, we have examined erythrocyte GSH, which is most potent endogenous antioxidant, plasma α- tocopherol and ascorbic acid , which are two most important nutrient antioxidants and TAA by FRAP assay which practically measures antioxidant strength contributed by nutrient and other antioxidants in the diet. It is interesting to note that smokers had the normal levels of erythrocyte GSH and plasma α-tocopherol and ascorbic acid but significantly lower levels of TAA ($p<0.05$). These three observations taken together point out that the dietary antioxidants other than α- tocopherol and ascorbic acid are consumed to scavenge RS in smoke. Plasma uric acid was raised in smokers and is in conformity with many other reports[29]. The rise in uric acid could be due to two reasons viz increased uric acid production and altered renal function. Since all the subjects had normal renal function as was apparent from their normal health and also by normal creatinine levels**,** the raised uric acid level should be due to increased uric acid synthesis consequent to enhanced conversion of xanthine dehydrogenase to xanthine oxidase[29]. Indeed, uric acid is a good antioxidant but can not be raised without reason as it is accompanied with raised FR production and has multiple other toxic effects. Xanthine oxidase is potent producer of superoxide anion ($O_2^{-\bullet}$) and amino carbonyl radical[30]. Uric acid is oxidative end product of purines which are essential component of nucleic acids. The rise in uric acid therefore also indicates enhanced breakdown and damage of nucleic acid including genetic material, whose implications could be reckoning factor in smoke induced toxicity[31,32]. The enhanced consumption of TAA could be partly due to scavenging of O_2 produced by xanthine oxidase activity. A comparison between same gender of smokers and never- smokers showed some distinct trends. Male smokers had only lower TAA as compared to never-smokers. The rest of indices were comparable. On the other hands female smokers had raised OS and uric acid and lower GSH and TAA as compared to female never-smokers. These findings imply that females are more prone to oxidative insult.

Lastly, Pearson's correlation coefficient was applied to ascertain some relevant relationships (table 3). The results allude: a. uric acid contributes to antioxidant activity both in smokers ($P<0.01$) and never-smokers ($P<0.05$) and rise in uric acid further supplements it but then uric acid production is also associated with enhanced production of FR; b. the OS and GSH levels being inversely related indicate that GSH is regularly and in all likelihood is proportionately consumed for maintaining redox balance and c. finally but most importantly the positive relationship between PTBARS and UTBARS ($P<0.05$) suggests that in smokers, kidneys have capacity to remove excess oxidative adducts proportionately. However, this capacity is limited only because in many conditions, PTBARS is considerably raised as kidneys are unable to stretch beyond its limit to excrete. No influence of smoking was seen in this cohort on blood sugar level or its relationship with OS.

In conclusion, our data on smokers indicate that smoking increases lipid peroxidation but body status remains unaffected as kidneys (possibly other routes also) excrete the excess peroxidation products formed; that dietary antioxidants, other than α-tocopherol and ascorbic acid are consumed to scavenge RS in smokes; that uric acid is raised and acts as an antioxidants but this does not qualify it to be useful owing to multifarious toxic effects including adverse results on genetic materials due to accompanied reactive oxygen species production. As a corollary, our data disagree with the hypothesis that RS in smoke are major contributors to its toxicity. This is indeed astonishing in view of amazing load of RS in smoke.

References

1. Mackay J and Eriksen M. The Tobacco Atlas, World Health Organization, Geneva; 2002.
2. Thun MJ. When truth is unwelcome: the first reports on smoking and lung cancer. Bull WHO 2005;83:144-45.
3. Pandey MR, Neupane RP and Gautam A. Epidemiological study of tobacco smoking behaviour among adults in a rural community of the hill region of Nepal with special reference to attitude and beliefs. Int J Epidemiol 1989;18:465.
4. Smoking and Health in Asia. WHO Chron 1982;36:156-59.
5. Rockville. Reducing the health consequences of smoking: 25 years of progress. A report of the Surgeon General, US Department of Health and Human Service, Centers for Disease Control, Center for Chronic Disease Prevention and Health promotion, Office on smoking and Health; 1989.
6. Singh PP, Gupta S, Barjatiya MK, Mamtha GP and Adhikari D. Oxidant antioxidant dovetail hypothesis: Let us not sprint before we stand. In: Singh PP, et al editors. Free Radicals and Antioxidants in Health and Disease. Chaudhary Offset Print, Udaipur,India, 2007:1-31.
7. Singh PP, Pendse AK, Bomb BS, Barjatiya MK and Ghosh R. Free radicals and antioxidants: sort out facts from fiction. In: Singh PP, et al editors. Free Radicals and Antioxidants. Chaudhary Offset Print, Udaipur,India,1999:15-19.
8. Cross CE, Traber MG, Eiserich J and Van der Vleit A. Micronutrient antioxidants and smoking. Brit Med Bull 1999;55:691-04.
9. Edward P, Morrow JD, Hudes M, Caan B, and Packer L. Factors associated with oxidative stress in human population. Am J of Epidem 2002:156.
10. Singh PP, Bomb BS, Ralot T and Jain S. Tobacco users are at greater risk of free radical injury-I. Oxidative stress and total antioxidant activity in bidi smokers and tobacco chewers. In: Singh PP, et al editors. Free Radicals and Antioxidants in Health and Disease: Concordance and Discordance. Chaudhary Offset Print, Udaipur,India,2007:61-70.
11. Aghwani T, Djalali M, Keshavari A, Sadeghi MR, Zevaati H and Negahdav M. Plasma levels of antioxidant vitamins and lipid peroxidation in breast cancer patients. Iranian J Publ Health 2006; 35(1):42 -47.
12. Adhikari D, Baxi J, Nagamma T, Azhari S and Singh PP. Vitamin C status in cancer patients and healthy subjects from the local population of Pokhara. Kath Univ Med J 2006;4:302-06.
13. Risal S, Adhikari D, Alurkar VM and Singh PP. Oxidative stress and antioxidant status in cardiovascular diseases in population of western Nepal. Kath Univ Med J 2006;4:271-74.
14. Adhikari D, Baxi J, Risal S and Singh PP. Oxidative stress and antioxidant status in cancer patients and healthy subjects, a case-control study. Nepal Med Coll J 2005;7:112-15.
15. Christine A, Northrop C, David I. and Thurnham. Monitoring micronutrients in cigarette smokers. Clinica Chimica Acta 2007;37:14-38.
16. Buetler E and Kelly BM. Improved method for the determination of blood glutathione. J. Lab Clin Med 1963;61:882.
17. Benzie IFF and Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of

"antioxidant power": The FRAP assay, Annal Biochem 1996; 239: 70-76.
18. Buege JA and Aust SD. The thiobarbituric acid assay. Methods in Enzymology 1978; 52: 306-10.
19. Baker H and Frank O. Clinical Vitaminology, Methods and Interpretation, Interscience Publishers, John Wiley and sons Incs, New York, 1968:172.
20. Natelson S. Techniques of Clinical Chemistry, 3rd ed. Charles, C Thomas, USA, 1971:288.
21. Pryor WA and Stone K. Oxidants in cigarette smoke: Radicals, hydrogen peroxide, peroxynitrate and peroxynitrite. Am NY Acad Science 1993; 686:12-28.
22. Bensasson RV, Land EJ and Truscott TG. Activated forms of oxygen. In: Excited States and Free Radicals in Biology and Medicine. Oxford Uni. Press, 1993:101-41.
23. Siu GM and Draper HH. Metabolism of malonaldehyde in vivo and in vitro. Lipids1982;17(5):125.
24. Merken VM, Hageman GJ, Annemie MW, Macro A Bast A and Wouters FM. Rehabilitation decreases exercise- induced oxidative stress in COPD. Am J Respir Crit Cave Med 2005;172:994- 01.
25. Jain S, Tarun R, Bomb BS and Singh PP. Tobacco users are at greater risk of free radical injury-II. Plasma ascorbic acid and alpha tocopherol in bidi smokers and tobacco users. In: Singh PP, et al editors. Free Radicals and Antioxidants in Health and Disease: Concordance and Discordance. Chaudhary Offset Print, Udaipur, India, 2007:71-82.
26. Lykkesfeldt J, Viscovich M and Poulsen HE. Plasma malondialdehyde is induced by smoking: a study with balanced antioxidant profiles. Brit J of Nutr 2004;92: 203- 06.
27. Yang SR, Chida AS and Bauter M. Cigarette smoke induces pro-inflammatory cytokine release by activation of NF and post translational modifications of histone deacetylase in macrophages. Am J Physiol Lung Cell Mol Physiol 2006;7:335-42.
28. Patel P. Beena, Pankaj M Shah, Upendra M Rawal and Prabhudas S. Patel. Tobacco, antioxidant enzymes, oxidative stress and genetic susceptibility in oral cancer.In: Singh PP, et al editors. Free Radicals and Antioxidants in Health and Disease. Chaudhary Offset Print, Udaipur, India, 2007:129-36.
29. Kristine Y. Lain, Nina M, Ness BR and Roberts JM. Effects of smoking on uric acid and other metabolic markers throughout normal pregnancy. J of Clin Endocrinol and Met 2005;90:5743-46.
30. Kanellis J, Feig DI and Johnson RJ. Does asymptomatic hyperuricemia contribute to the development of renal and cardiovascular disease? An old controversy renewed. Nephrology 2004;9:394-99.
31. Armlle Munia, Stefano B, Anna V, Roberto Q, Monica N and Marco P. Bronchial malondialdehyde DNA adducts, tobacco smoking and lung cancer. Free Rad Biol and Med 2006;41:1499-05.
32. Maura L, Cristna L, Carlotta DF, Chiara R and Franco B. Smokers and passive smokers gene expression profiles: Correlation with the DNA oxidation damage. Free Rad Biol and Med 2007;43:415-22.