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RESEARCH ARTICLE

Effect of wheatgrass juice on lipid peroxidation , SOD activity and Catalase activity in lung cancer during chemotherapy.

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Key words:-

Abstract

A High level of oxidative stress is related with different cancer including lung cancer. Lipid peroxidation, Superoxide dismutase enzyme activity and Catalase enzyme activity are the important markers of oxidative stress. In the present study significantly increased levels of Lipid peroxidation in lung cancer patients were noticed in lung cancer patients before chemotherapy (Lipid peroxidation (**6.44 ± 0.63)**, Superoxide dismutase **(1.03 ± 0.14)**, Catalase (**4.77±0.37)** and after chemotherapy (Lipid peroxidation (**7.31 ± 0.44)**, Superoxide dismutase (**0.81 ± 0.14)** , Catalase **(3.89±0.27**) as compared to healthy controls (Lipid peroxidation (**2.11 ± 0.35)** , Superoxide dismutase **(1.79±0.30)** , Catalase (**8.10±0.42**) which shows increased oxidative stress in lung cancer patients. The levels of oxidative stress were found to be increased during chemotherapy. The levels of oxidative stress were found to be decreased in lung cancer patients before chemotherapy (Lipid peroxidation (**6.35 ± 0.43)**, Superoxide dismutase (**0.99 ± 0.22)**, Catalase (**4.66±0.44**) and after chemotherapy (Lipid peroxidation (**5.84 ± 0.67)**, Superoxide dismutase (**0.87 ± 0.12)**, Catalase (**4.18±0.31**) receiving wheatgrass juice during chemotherapy. It can be conclude from the present study that increased oxidative stress is present in lung cancer patients, which further increases during chemotherapy and wheatgrass juice can act as a natural antioxidant supplement during chemotherapy to decrease oxidative stress and in effectiveness of the chemotherapy drugs.

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Introduction:-

Lung cancer is known as the highest cause of cancer mortality all around the world in both men and women (Parkin et al., 2005). The incidences of lung cancer are rising every year by 0.5% which results in about 1.04 million new patients of lung cancer every year worldwide (Maas et al., 2007). Cigarette smoking is the main cause of lung cancer in most of the cases, a part from it some other factors such as radioactive gases, heredity and breathing in toxic chemicals also leads to lung cancer. A part from it some others factors like age, race, gender, occupation, air pollution, radiation , diet, viral infections, leads to only 6% development of lung cancer (Spiro SG and Porter JC,

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2002). Ciggarette smoke contains about 4000 chemicals in which about 60 can cause cancer (Hecth, 1999). Polycyclic aromatic hydrocarbon (PAH) in addition with nitrosamines, aromatic amines, aldehydes, volatile organic compounds, oxidants and metals ae main carcinogens present in cigarette smoke (Hecht, 2006). A large amount of reactive oxygen species (ROS) like superoxide, hydrogen peroxide, hydroxyl and peroxyl radicals are present in a single puff of cigarette smoke which are generally responsible for inducing lung cancer (Church DF and Pryor WA, 1985; Hecht S, 2007).

Lipid peroxidation is a chain reaction in which oxidation of polyunsaturated fatty acids in plasma membrane takes place due to free radicals. Lipid peroxidation is an important indicator of oxidative cell damage. (Pryor WA and Godber SS, 1991). Any species with sufficient reactivity to abstract a hydrogen atom (H.) from a methylene group (-CH_2-) can cause lipid proxidation, because hydrogen atom contains only one electron, abstraction leaves behind an unpaired electron on the carbon, -CH-. The presence of double bond in the fatty acid weakens the C-H bonds on the carbon atom adjacent to the double bond and thus facilitates H^+ removal. Hence the polyunsaturated fatty acids side chains of membrane lipids are particularly sensitive to peroxidation. Reactive oxygen species such as Superoxide (O_2.) hydrogen peroxide (H_2O_2), hydroxyl (OH.) and peroxyl (ROO.) radicals can easily cause lipid peroxidation. (Gutteridge, 1988). It is cleared by a research that the lung cancer patients have high MDA level (Malondialdehyde) which is one of the end product of lipid peroxidation, and also having low levels of COQ10 which act as an electron carrier in ETC to produce ATP (Honda et al., 2000; Erhola et al ., 1997)

Superoxide dismutases (SOD), Catalase and Glutathione peroxidase are the main components of enzymatic antioxidant defense system of the cell. Superoxide dismutase catalyze the dismutaion of more harmful superoxide molecule in to less harmful hydrogen peroxide molecule and oxygen.

$$2\ O_2^{\cdot -} + 2\ H^+ \Longrightarrow H_2O_2 + O_2$$

Another antioxidant enzyme Catalase is found in nearly all living organisms exposed to oxygen catalyzes the composition of hydrogen peroxide to water and oxygen. (Chelkani P et al., 2004). Increased levels of oxidative stress (elevated plasma MDA levels) were not only found in lung cancer patients but also in some other lung related disease such as tuberculosis (Kwaitkowska G et al., 1999). Oxidative stress is found in both lung cancer patients and tuberculosis patients, but the high extent of lipid peroxidation and extreamly low levels of SOD (an antioxidant enzyme) is present in lung cancer patients as compared to tuberculosis patients. (Yildiz Gunney et al., 2004). Gupta et al conducted a study on non-small cell lung cancer patients found high levels of LPO and NO and low levels of GSH and SOD in lung cancer patients as compared to the healthy subjects. After the third and sixth cycle of chemotherapy the levels of LPO and NO increased and levels of GSH and SOD decreased. It shows that in lung cancer patients oxidative stress increased and antioxidant enzymes decreased with the progression of the disease. (Gupta el al., 2010).

Materials and methods:-

Blood samples of 100 lung cancer patients who were admitted to Cancer Hospital and Research Institute Gwalior (M.P.) and 50 healthy subjects were collected. Lung cancer patients were divided in two groups, group 1^{st} received only chemotherapy and group 2^{nd} received 50 ml of wheatgrass juice daily during chemotherapy. Blood samples were collected from both the groups of lung cancer patients before the chemotherapy and after the 3^{rd} cycle of chemotherapy.

Sample collection and processing:-

The blood samples were drawn from the antecubital vein nenopuncture. The blood was collected in tubes containing EDTA (an anticoagulant, 2mg/ml) and sodium fluoride (an inhibitor of glycolysis, 2mg/ml). Each blood sample was centrifuged for 10 min at 3000rpm. The plasma was collected and stored at -20^0 C, which is used for further investigation.

Lipid peroxidation is measured by method of Ohkawa et al., 1979. Haemolysate SOD activity was assayed by the method of Winterbourn et al.,1975. Catalase activity was assayed following the procedure of Sinha, (1972).

Results and Discussion:-

Table-1. Level of oxidative stress markers lung cancer patients before and after chemotherapy.

Oxidative stress markers	Before chemotherapy (0 cycle) N= 50	After 3 cycles of chemotherapy N= 36	Percentage (%) change	Healthy controls
Lipid peroxidation	**6.44 ± 0.63[a]**	**7.31 ± 0.44[ab]**	**11.90%**	**2.11 ± 0.35**
Superoxide Dismutase (SOD)	**1.03 ± 0.14[a]**	**0.81 ± 0.14[ab]**	**21.35%**	**1.79±0.30**
Catalase (CAT)	**4.77±0.37[a]**	**3.89±0.27[ab]**	**18.44%**	**8.10±0.42**

Values are expressed mean± SD
TBARS:µg/ml; SOD: units/min/mg protein;
Catalase; units/min/mg protein
[a] P<0.01 in superscript showed comparison of controls group with patients groups and were significant at P<0.01
[b] P<0.01 in superscript showed comparison of patients group with patients groups (after 3 cycle) and were significant at P<0.01

Table-2. Levels of oxidative stress markers lung cancer patients before and after chemotherapy with wheatgrass juice.

Oxidative stress markers	Before chemotherapy (0 cycle) N=50	After 3 cycles of chemotherapy N= 44	Percentage (%) change
Lipid peroxidation	**6.35 ± 0.43[a]**	**5.84 ± 0.67[a]**	**10.56% decrease from initial levels.**
Superoxide Dismutase (SOD)	**0.99 ± 0.22[a]**	**0.87 ± 0.12[ab]**	**12.12%**
Catalase (CAT)	**4.66±0.44[a]**	**4.18±0.31[ab]**	**10.30%**

Values are expressed mean± SD
TBARS:µg/ml;SOD: units/min/mg protein;
Catalase; units/min/mgprotein
[a] P<0.01 in superscript showed comparison of controls group with patients groups and were significant at P<0.01
[b] P<0.01 in superscript showed comparison of patients group with patients groups (after 3 cycle) and were significant at P<0.01

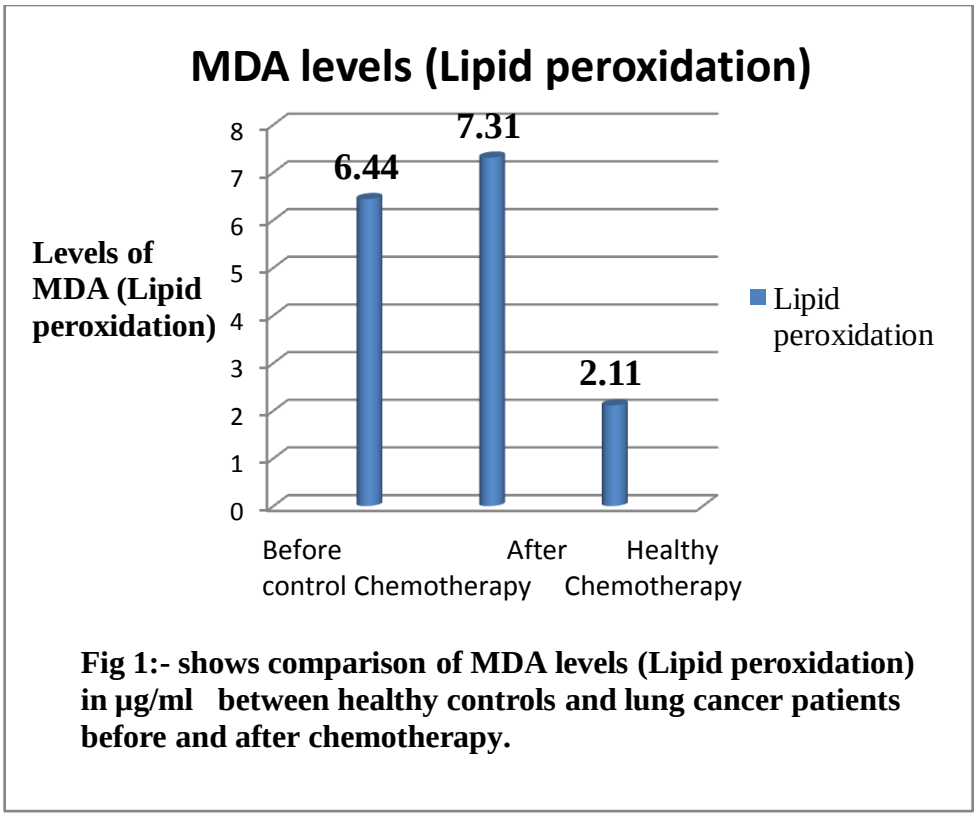

Fig 1:- shows comparison of MDA levels (Lipid peroxidation) in µg/ml between healthy controls and lung cancer patients before and after chemotherapy.

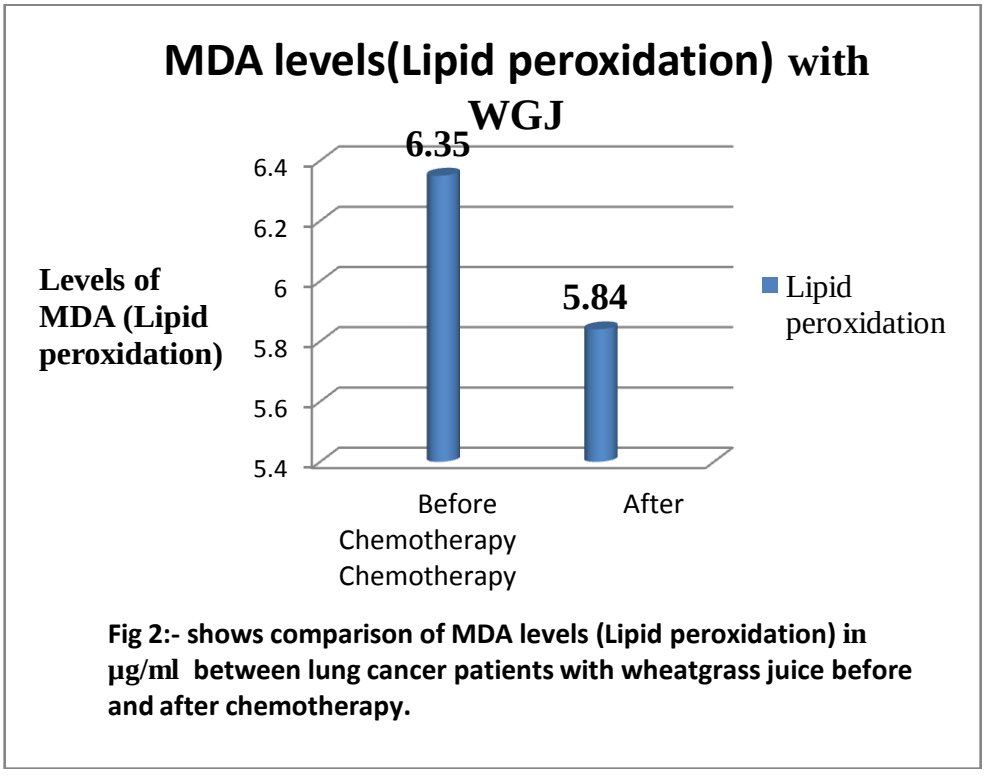

Fig 2:- shows comparison of MDA levels (Lipid peroxidation) in µg/ml between lung cancer patients with wheatgrass juice before and after chemotherapy.

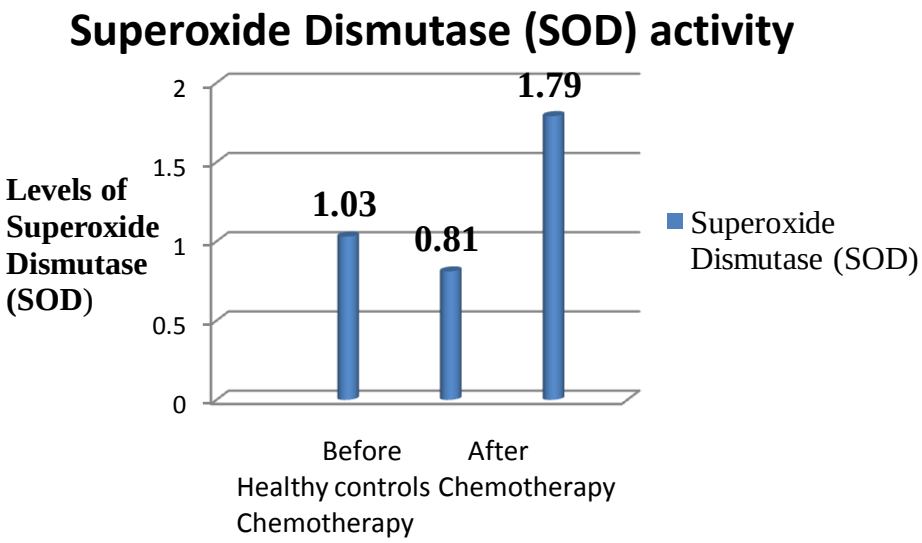

Fig 3:- shows comparison of SOD enzyme activity in units/min/mg protein between healthy controls and lung cancer patients before and after chemotherapy.

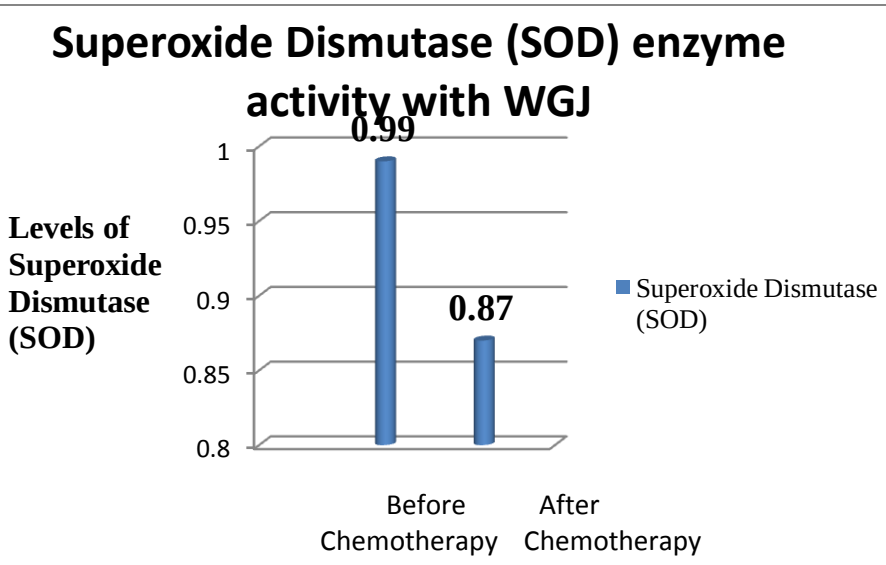

Fig 4:- shows comparison of SOD enzyme activity in units/min/mg protein between lung cancer patients before and after chemotherapy with wheatgrass juice.

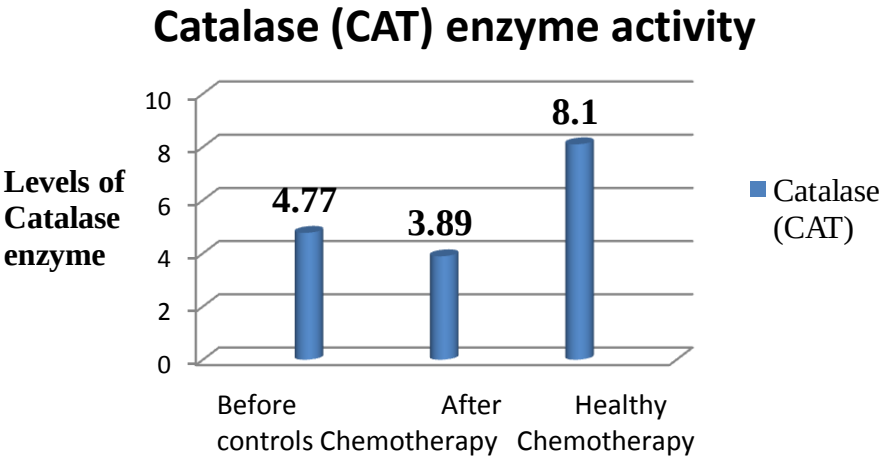

Fig 7:- shows comparison of Catalase enzyme activity in units/min/mg protein in lung cancer patients before and after chemotherapy with Healthy controls.

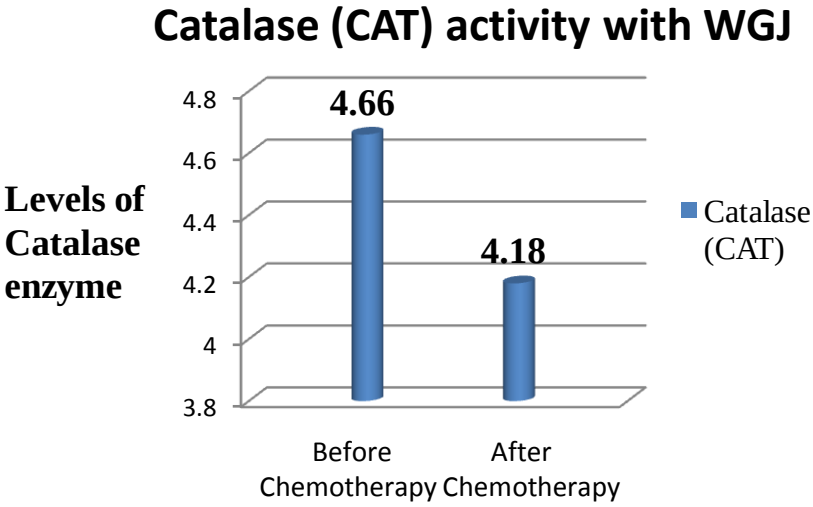

Fig 8:- shows comparison of Catalase enzyme activity in units/min/mg protein in lung cancer patients before and after chemotherapy with wheatgrass juice.

In the present study the levels of lipid peroxidation, superoxide dismutase activity and catalase activity were calculated before and after chemotherapy in two groups of lung cancer patients group 1st received only chemotherapy and group 2nd received 50 ml of wheatgrass juice daily during the 3 cycles of chemotherapy and the values were compared between group 1st and 2nd and also compared with the healthy controls. Values of lipid peroxidation, superoxide dismutase and catalase were found to be (**2.11 ± 0.35), (1.79±0.30)** and (**8.10±0.42)** respectively in healthy controls. Significantly higher values of lipid peroxidation **(6.44 ± 0.63),** and lower values of superoxidase dismutase**(1.03 ± 0.14)** and catalase **(4.77±0.37)** were found in lung cancer patients as compared to healthy controls which shows high oxidative stress in lung cancer patients. The values of lipid peroxidation**7.31 ± 0.44**, increases significantly and superoxide dismutase activity and catalase activity significantly decreases after the 3 cycles of chemotherapy in group 1 patients. The levels of lipid peroxidation were found to be decreased in group 2nd lung cancer patients with wheatgrass juice from **6.35 ± 0.43** to **5.84 ± 0.67**, Superoxide dismutase activity and Catalase activity were found to be decreased from **0.99 ± 0.22** to **0.87 ± 0.12** and **4.66±0.44** to **4.18±0.31** respectively after 3 cycles of chemotherapy. The above results shows that wheatgrass juice a rich source of antioxidant can reduce oxidative stress during chemotherapy in lung cancer and increase the effectiveness of chemotherapeutic drugs.

Reference:-

1. Chelkani, P., Fita, I., Loewen, PC. (2004). "Diversity of structures and properties among catalases" cell. mol. life sci. 61 (2):192-208.
2. Church, DF., Pryor, WA. (1985). Free radical chemistry of cigarette smoke and its toxicological implications. Envioron HLTH prespect. 64: 111-26.
3. Erhola, M., Toyokuni, S., Okada, K. (1997). Biomarker evidence of DNA oxidation in lung cancer patients: association of urinary 8-hydroxy-2'-deoxyguanosine excretion with radiotherapy, chemotherapy and response to treatment. FEBS Lett. 409: 287-91.
4. Gupta, A., Srivastava, S., Prasad, R., Natu, SM., Mittal, B., Negi, MP., Srivastava, AN. (2010). Oxidative stress in non-small cell lung cancer patients after chemotherapy: association with treatment response. Respirology. 15(2): 349-56.
5. Gutteridge, JMC. (1988). Lipid peroxidation; some problems and concepts. In;Halliwell B,ed. Oxygen radicals and tissue injury. Betheads. MD; FASEB: 9-19.
6. Hecht, SS. (1999): Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst 91: 1194-210.
7. Hecht, SS. (2006): Smoking and lung cancer-a new role for an old toxicant? Proc Natl Acad Sci USA 103:15725-26.
8. Hecht, S. (2007). Cigarette smoking and lung cancer: chemical mechanism and approaches to prevention. Lancet oncol. 3:461-9).
9. Honda, M., Yamada, Y., Tomonaga, M., Ichinose, H., Kamihira, S. (2000). Correlation of urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage, and clinical features of hematological disorders: a pilot study. Leuk Res. 24:461-68.
10. Kwiatkowska, G., Piasecka, MZ., piotrowta, W., Nowata, D. (1999) Increased serum concentrations of conjugated diens and malondialdehyde in patients with pulmonary Tb. Resp med. 93:272-6.
11. Maas, KW., EI Sharouni, SY., Smit, EF., Schramel, FM. (2007). Sequencing chemotherapy, radiotherapy and surgery in combined modality treatment of stage III non small cell lung cancer, Curr Opin Pulm Med. 13: 297-304.
12. Ohkawa, H,, Ohishi, N., Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 95: 351-8.
13. Parkin, DM., Bray, F., Ferlay, J., Pisani, P. (2005). Global cancer statistics, 2002, CA Cancer J Clin.55: 74-108.
14. Pryor, WA., Godber, SS. (1991) Non invasive measures of oxidative stress status in humans. Free Rda Biol Med. 10:177-184.
15. Sinha, KA. (1972). Colorimetric assay of catalase. Anal. Biochem. 47: 389-94.
16. Spiro, SG., Porter, JC. (2002). Lung cancer, where are we today? Current advances in staging and nonsurgical treatmen. Am j Respir crit care Med. 166: 1166-96.
17. Winterboun, CC. (1975). Hawking RE, Brain M., and Carrel RW. J. Lab. Clin. Med. 2: 337-341.
18. Yildiz. G., Ayse, B., Ciftci, TU., Filiz, C., Ozgur, C. (2004). Serum malondialdehyde levels and superoxide dismutase activities in pulmonary tuberculosis and lung cancers. Ankara Üniversitesi Dikimevi Saglık Hizmetleri Meslek Yüksekokulu Dergisi, Cilt 6, Sayı 2.