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The Effects of Tobacco Harvesting on Oxidant-Antioxidant Status, Some Biochemical and Hematologic Markers in Women Workers

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Abstract: The aim of the study was to evaluate the effects of tobacco harvesting on oxidant-antioxidant status, as well as its biochemical and hematological markers evaluation in women workers. Blood Malondialdehyde (MDA) level, Superoxide Dismutase activity (SOD), plasma Zinc (Zn) concentration, ALT and AST enzyme activity and some hematological parameters were evaluated in women workers (n = 20). Two blood samples were taken from each worker, the first of which was taken in the morning before breakfast, at the day before the first harvest in the 1st week of July and the second, just after the 50 days harvesting period procedure at the end of August. The blood MDA levels increased significantly after harvesting. The mean SOD activity following harvesting season was significantly lower. Whereas, blood Zn levels of groups didn't show any significant change. After harvest season red blood cell, Hb and Htc levels were found to be significantly decreased compared before harvest season. The ALT concentrations were significantly increased after harvesting compared to before harvesting. Although, AST hand concentrations were significantly decreased after harvesting compared to before harvesting. Consequently, tobacco harvesting is a stressful procedure and induced oxidative stress in blood by decreasing the activities of antioxidant enzymes and generation of free radicals in women workers. Moreover, the antioxidant treatments could provide great advantages for workers to avoid from oxidative stress and maintain a healthy life span.

Key words: Tobacco harvesting, toxication, oxidative stress, MDA, SOD, women worker

INTRODUCTION

One of the focused points of the today's scientific world, the health risks associated with smoking tobacco and exposure to secondhand smoke are well known. Less well known are the health effects of handling wet tobacco leaves (McBride *et al*., 1998). Green Tobacco Sickness (GTS) is a form of nicotine poisoning that affects workers who have direct contact with tobacco plants during cultivation and harvesting (Gehlbach *et al*., 1974). Tobacco cultivation is seasonal and hazardous cultivation practices last for 2.3 months during the harvesting season. Tobacco cultivation involves different processes such as harvesting of plants and leaves, separation of leaves, stringing and tying of leaves before they are kept in a barn for curing, grading, etc. The agricultural practices followed during tobacco cultivation, which lead to the smearing of thick, gummy plant sap on the hands of workers and other parts of their bodies that come in contact with tobacco leaves. This leads to the absorption of nicotine through the dermal route. Workers engaged in various processes get cuts and abrasions on their palms and the skin around their nails gets peeled off, facilitating nicotine absorption (Reddy and Gupta, 2004). Once nicotine is absorbed, it is distributed throughout the body, including into the brain. Nicotine excites sensory nerves from the gut and parasympathetic nerves in the gastrointestinal tract, which lead to an overall increase in gastrointestinal secretion and motility. The pharmacological effects of nicotine on nicotinic receptors in the central nervous system and at post-synaptic autonomic ganglia have been well elaborated and help to explain the toxic effects of nicotine (Taylor, 1996). According to our knowledge, there are some studies regarding GTS (Gehlbach *et al*., 1974), but a limited number of studies about the effect of tobacco harvesting on oxidant-antioxidant balance in women workers.

Overwhelming evidence indicates that oxidative stress can lead to cell and tissue injury. However, the same free radicals that are generated during oxidative

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stress are produced during normal metabolism and thus are involved in both health and disease (Packer *et al*., 2004). Under normal circumstances, the generated Reactive Oxygen Species (ROS) are detoxified by the antioxidants present in the body and there is equilibrium between the generated ROS and the present antioxidants. However, owing to ROS overproduction or inadequate antioxidant defense, this equilibrium is hampered favouring the ROS upsurge that culminates in oxidative stress. The ROS readily attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA (Farber, 1994; Kaur *et al*., 2000). The oxidative damage is a crucial etiological factor implicated in several chronic diseases such as cancer, atherosclerosis, arthritis, neurodegenerative diseases and also in the ageing process (Fidan *et al*., 2008).

The objective of this study was to to evaluate the effect of tobacco harvesting on antioxidant defense systems, lipid peroxidation, some biochemical and hematologic parameters in women workers. This research is also important for being to first research that evaluates the parameters that mentioned about tobacco harvesters in Aegean area and Turkey.

MATERIALS AND METHODS

Subjects and experimental design: The study was conducted in Usak, is a city, which plays a central role in tobacco production in Turkey and 20 (n = 20) woman workers (35±10) were enrolled in the study. Subjects are chosen as at least 5 years tobacco harvesters. Admission criteria for the workers were as follows: no pregnancy, no smoking, no alcohol and no bacterial infection or other diseases.

Two blood samples were taken from each workers, the first of which was taken in the morning before breakfast, at the day before the first harvest in the 1st week of July and the second just after the harvesting period (50 days) procedure at the end of August. Sun rays did not affect the research because harvesting lasts from midnight to sun shine and also season did not affect because harvesting was done in summer.

Blood samples were taken from brachial vein into heparinized tubes for Measured Malondialdehyde (MDA), Superoxide Dismutase activity (SOD), plasma Zinc (Zn) concentration, ALT and AST enzyme activity, Red Blood Cell (RBC), Hemoglobin (Hb) and Hematocrit (Htc). Lipid peroxidation was determined by measuring the MDA concentration. The blood MDA levels, an index of lipid peroxidation, were measured by the double heating method of Draper and Hadley (1990). SOD activity was measured by using the method described by Marklund (1990). Zinc concentration was estimated by the Nitro-PAPS method using a commercially available kit obtained from Chema Diagnostica, Italy (catalog number: ZN 0125 CH). Measures of biochemichal parameters were made by I Lab 1800 autoanalyzer and blood cell count was calculated by LabAdmin autocell counter.

Statistical analysis: All data were presented as mean±SE. The comparisons of parameters were performed with paired t-test. Data were analyzed using the SPSS® for Windows computing program (version 10.0) and $p<0.05$ was considered statistically significant (Sokal and Rohlf, 1969).

RESULTS AND DISCUSSION

The effect of tobacco harvesting on the blood SOD, Zn contents and MDA production as an estimate for lipid peroxidation induced by tobacco harvesting is shown in Table 1, 2 and Fig. 1.

As shown in Table 1, the amount of MDA in experimental group after harvesting, was found to be increased compared to before harvesting ($p<0.05$), while the mean SOD activity following harvesting season was significantly lower ($p<0.05$). Whereas, blood Zn levels of groups didn't show any significant change.

In this study, after harvest season red blood cell, hemoglobin and hematocrit levels were found to be significantly decreased compared before harvest season ($p<0.05$).

The interest in ROS in biology and medicine has been increased because of their strong relationship with phenomena such as aging and disease processes (Cao *et al*., 1995). Active oxygen species and oxygen radicals are common products of cellular metabolism. However, excessive generation of free radicals can occur due to endogenous biological or exogenous environmental factors, such as exposure to radiation, pollution or chemical substances (Fidan *et al*., 2008). A cell defends itself against ROS by elaborating systems of biological defense. The antioxidant defense system includes small molecular antioxidants, antioxidant enzymes and metal chelating agents (Vidyasagar *et al*., 2004). Enzymatic scavengers like Superoxide Dismutase (SOD), protect the system from deleterious effects of reactive oxygen species and pesticides have been reported to cause alteration in antioxidants or free radical scavenging system (Oberoi *et al*., 2007). In spite of numerous biological defense systems, increased free radical generation has the potential to result in oxidative

Table 1: Effects of tobacco harvesting on MDA, SOD and Zn contents in women workers

Parameters	Before harvesting $\bar{x} \pm SE$	After harvesting $\bar{x} \pm SE$
MDA (nmol mL^{-1})	9.99±0.590	13.23±0.710*
SOD (U mL^{-1} blood)	376.67±95.22	244.36±90.95*
Zn (μg dL^{-1})	12.89±1.068	12.26±1.100

Table 2: Effects of tobacco harvesting on RBC, Hb and Htc levels in women workers

Parameters	Before harvesting $\bar{x} \pm SE$	After harvesting $\bar{x} \pm SE$
RBC (10^6 μL^{-1})	4.41±0.10	3.66±0.23*
Hb (g dL^{-1})	12.48±0.29	9.95±0.28*
Htc (%)	37.18±0.72	29.77±0.92*

*p<0.05

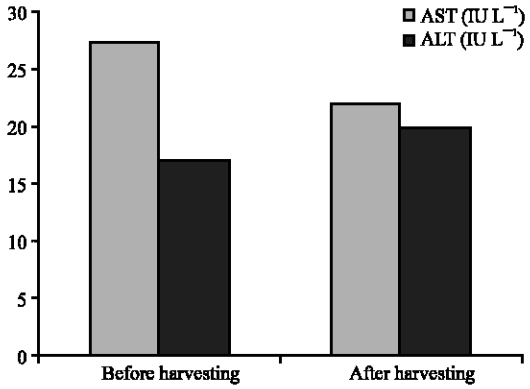

Fig. 1: The ALT concentrations were significantly increased after harvesting compared to before harvesting (p<0.05). Although, AST hand concentrations were significantly decreased after harvesting compared to before harvesting (p<0.05)

stress. Oxidative stress may result from an imbalance between ROS and antioxidants levels (Lightboy *et al*., 2001). It is well known that when the organism cannot balance free radical generation with the defense systems, a cellular injury and tissue damage or cell death might occur. The impact made by free radicals on lipids is named as Lipid Peroxidation (LP). Since, membrane phospholipids are the major targets of oxidative damage, lipid peroxidation is often the first parameter analyzed for proving the involvement of free radical damage. Thus, the presence of MDA is considered as an indicator of free-radical damage through membrane lipid peroxidation (Katz *et al*., 1996; Enginar *et al*., 2006).

Many commonly used chemicals in the workplace or home have the potential to cause systemic toxicity if they are absorbed through the skin. Each chemical exposure to the skin will result in a different risk of systemic toxicity, depending on the characteristics of the exposure and the inherent characteristics of the chemical (McDougal *et al*., 2007). Farm workers generally are exposed to a combination of synthetic agricultural chemicals and natural products while working in the fields (Dowla *et al*., 1996). It is possible that tobacco field workers are exposed to higher levels of agricultural chemicals than other field workers because working with tobacco involves close contact with the tobacco plants, while topping, cutting and then hanging them for drying after the plants are sprayed with the agricultural chemicals such as pesticide (Dowla *et al*., 1996). Tobacco farmers had a risk of nicotine and pesticide poisoning from tobacco leaves (Weizenecker and Deal, 1970; Gehlbach *et al*., 1974). Once absorbed, nicotine is extensively metabolized by the liver to a number of major and minor metabolites (Snyder *et al*., 1993; Cashman *et al*., 1992; Neurath, 1994).

Metabolism of nicotine to cotinine *in vivo* occurs within minutes after nicotine absorption, with cotinine having a half-life of 19-24 h in rodents (Sastry *et al*., 1995). Nicotine is also converted to a number of biologically important compounds during harvesting and fermentation of tobacco (Brunneman *et al*., 1996). On the other hand, nicotine also induces oxidative stress both *in vivo* and *in vitro* that causes a peroxidant/antioxidant imbalance in blood cells, blood plasma and tissues (Suleyman *et al*., 2002). Further more, pesticides may induce oxidative stress leading to generation of free radicals and alteration in antioxidant or oxygen free radical scavenging enzyme system (Fidan *et al*., 2008). Guan *et al*. (2003) reported that mitocondrial reperatory chain is broken by nicotine and cause an increase the level of superoxide and hydrogen peroxide. Furthermore, high dose nicotine caused an oxidative stress by increasing levels of cytP450 (Newman *et al*., 2002). Clara *et al*. (2001), determined that nicotine deteriorates the antioxidant defense wall by increasing the oxidative stress. There are many studies that declare the pathophysiology of nicotine-induced organ damage, which is related with the increase in lipid peroxidation (Yildiz *et al*., 1999, 1998). Ashakumary and Vijayammal (1996) demonstrated that nicotine promotes atherogenesis by increasing lipid peroxidation in a rat model. Increase in lipid peroxidation has been reported in nicotine-administered rats and in pancreatic tissue, esophageal mucosa and Chinese hamster ovary cells when incubated with nicotine (Sheng *et al*., 2001). Dündar *et al*. (2001), reported that lipid peroxidation increased in tobacco workers compared the control subjects.

In present study, the amount of blood MDA in experimental group after harvesting, was found to be increased compared to before harvesting, which is in parallel to literature reports. On the other hand, various chemicals, including nicotine, have been shown to cause cellular damage by affecting the cellular antioxidant

defense systems (Sheng *et al*., 2001). Pathogenesis of atherosclerosis in nicotine-administered rats is associated with a decrease in SOD activity (Ashakumary and Vijayammal, 1996). Addition of SOD enzyme to nicotine-treated tissues *in vitro* has been shown to dampen the effects of lipid peroxidation (Sheng *et al*., 2001). Our data showed that SOD levels following harvesting season were depressed. In this context, it is possible that the observed insufficiency in antioxidant activity could be due to direct modification of the antioxidant defenses by tobacco harvesting.

Serum zinc is probably the most commonly used indicator but it rapidly decreases in the presence of inflammation. Inflammation produces a systemic response that includes hypozincemia (Beisel, 1976). In general, the differences reported between smokers and non-smokers in serum zinc concentrations reflect the duration and amount of smoking exposure. Kocyigit *et al*. (2001) analyzed plasma zinc concentrations and erythrocyte CuZn-SOD activities in 58 apparently healthy men of whom 26 had never smoked and 32 had smoked N10 cigarettes/day for at least 1 year. Plasma zinc concentrations were unaffected by smoking, but CuZn-SOD activity was higher in the smokers. Similarly, hypozincemia was not present in teenage Korean girls who had smoked N10 cigarettes/day for at least 1 year (Kim *et al*., 2003). In this study, the blood Zn levels of experimental groups in our study didn't show any significant change.

In this study, we observed an increase in ALT enzyme concentration and decrease AST, red blood cell, hemoglobin and hematocrit levels of women harvesting workers at the end of 50 days harvesting. Sagi and Fluth (2001) reported some changes in hemoglobin, erythrocyte and leukocyte levels of farm workers and they addressed the cause of these changes to the chemicals, viruses, bacteria, insecticide and residues in green tobacco leaves.

CONCLUSION

It was concluded that tobacco harvesting induced oxidative stress in blood by decreasing the activities of antioxidant enzymes and generation of free radicals in women workers. Therefore, the clinical trials of the antioxidants could provide great advantages to patients suffering from toxications tobacco harvesting and maintain life span of workers. On the other hand, it was believed further studies should be carried out to determine the relationship between tobacco harvesting and AST.

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