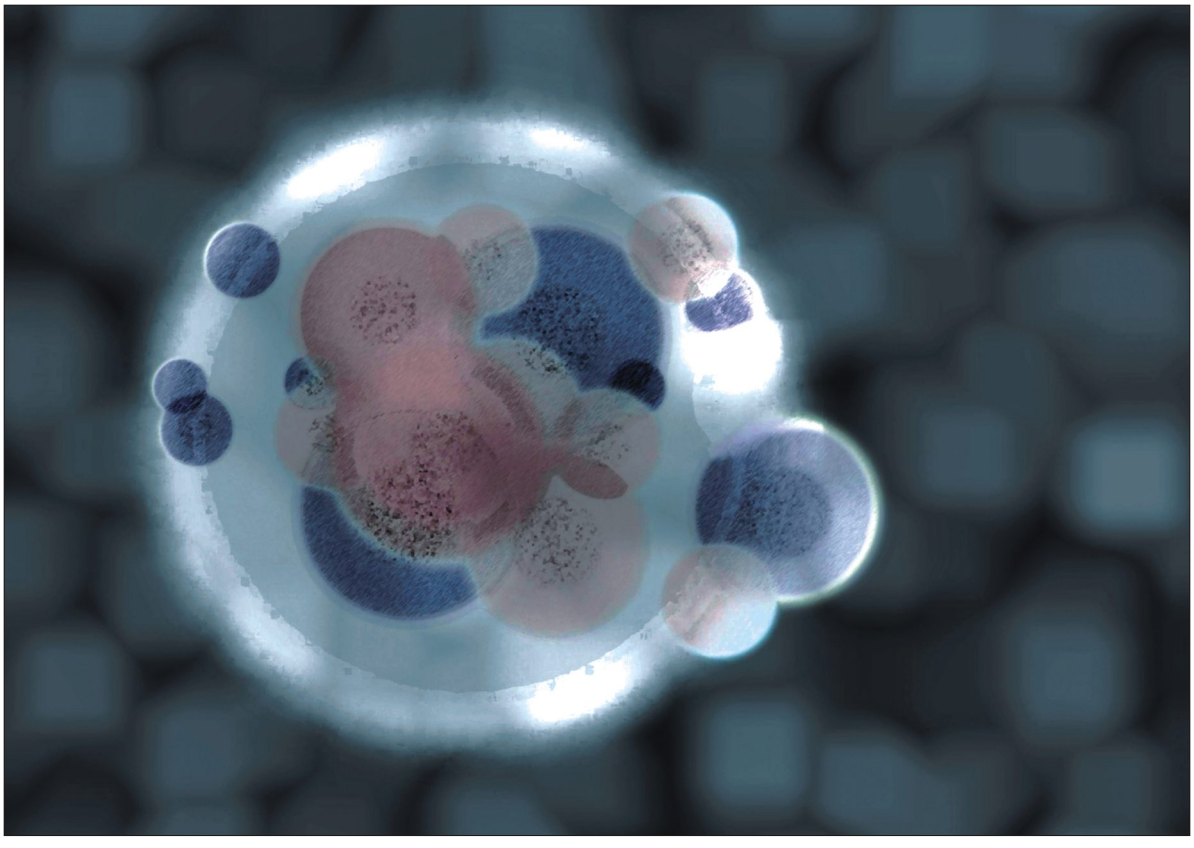

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2018.450.457

Research Article
Synergistic Oxidative Effects of Smoking and Pesticides Exposure on Reproductive Male Sex Hormones

[1]Amal Saad-Hussein, [2]Mohga Shafik Abdallah, [1]Eman Mohamed Shahy, [2]Manar Seleem Fouda and [2]Asmaa Mohammed Mohammed Abdel-Aleem

[1]Department of Environmental and Occupational Medicine, National Research Centre, El-Buhouth St. Dokki, Postal code 12622 Cairo, Egypt
[2]Department of Chemistry, Faculty of Science, Helwan University, Cairo, Egypt

Abstract

Background and Objective: Several studies indicate that pesticide intoxication produces oxidative stress. This oxidative stress plays an important role for human health and contributes significantly to many diseases and other chronic health effects. This study aimed to determine the role of oxidative stress of pesticides occupational exposure and smoking on male reproductive hormones and the antagonistic role of antioxidant enzymes. **Materials and Methods:** Follicle Stimulating Hormone (FSH), Luteinizing hormone (LH) and testosterone, Malondialdehyde (MDA), Superoxide dismutase (SOD), reduced glutathione (GSH), Glutathione S-transferase (GST) and Glutathione peroxidase (GPx), acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) were estimated in 51agriculture workers occupationally exposed to pesticides and 50 controls. **Results:** Results revealed significant decrease in AChE, BuChE, GSH and SOD levels in the workers compared to controls and significant increase in MDA, GPx and GST. Moreover, MDA and FSH were significantly increased among the smoker workers compared to non-smoker workers. The MDA was significantly correlated with GST and inversely correlated with AChE and BuChE in exposed workers. The FSH and LH were significantly correlated with SOD. **Conclusion:** Occupational exposures to pesticides and smoking have synergistic oxidative effect on FSH. It seemed that SOD and GST play important roles in antagonizing this oxidative stress among the examined pesticides workers. Therefore, smoking cessation and improving in the dietary habits with antioxidant supplementation could be essential for protection against the chronic toxic effects of occupational exposure to pesticides on the male reproductive hormones among smokers.

Key words: Pesticide sprayers, smokers, male sex hormones, oxidative stress, antioxidants, pesticide intoxication, male reproductive hormones

Received: April 08, 2018 Accepted: September 03, 2018 Published: October 15, 2018

Citation: Amal Saad-Hussein, Mohga Shafik Abdallah, Eman Mohamed Shahy, Manar Seleem Fouda and Asmaa Mohammed Mohammed Abdel-Aleem, 2018. Synergistic oxidative effects of smoking and pesticides exposure on reproductive male sex hormones. J. Biol. Sci., 18: 450-457.

Corresponding Author: Eman Mohamed Shahy, Department of Environmental and Occupational Medicine, National Research Centre, El-Buhouth St. Dokki, Postal code 12622 Cairo, Egypt Tel: 00201005263938

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Environmental Protection Agency (EPA) defines pesticides to be substances or mixture of substances/chemicals intended to prevent, destroy, repel or mitigate any pest[1]. Although pesticides usage has great benefits, it has several health risks associated to non-target subjects including humans who are occupationally or environmentally exposed to those compounds[2]. Such health risks occur among occupational exposed workers from improper handling of these hazardous compounds, mainly during mixing and loading of the equipment and improper application during spraying or harvesting sprayed crops[3].

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities have been widely used for monitoring exposure to organophosphorus (OP) and carbamate (CB) pesticides. In exposed populations, strong associations were detected between the reduction in AChE activities and exposure to those compounds and symptoms of chronic pesticides toxicity[4]. The acute adverse effects of OP on the central nervous system were mainly due to the inhibition of AChE at nerve endings, which leads to the accumulation of the neurotransmitter acetylcholine and consequently over-stimulation of muscarinic and nicotinic receptors[5].

In experimental animals, OP pesticides proved to act as endocrine disrupting chemicals (EDC), because of their capacity to impair the male sexual hormone profile[6,7]. Furthermore, epidemiological studies found that environmental or occupational exposure to OP may affect the male hormone profile[8,9]. Slimani *et al.*[10] observed that pesticides are responsible for decreasing testosterone concentrations either by inhibiting release of FSH or LH. Oxidative stress was proved to be induced by pesticides, either by over production of free radicals or by alteration in antioxidant defense mechanisms, including detoxification and scavenging enzymes[11,12]. The levels of the activities of antioxidant enzymes such as superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx) as well as the membrane lipid peroxidation (LPO) has been widely studied in order to ascertain the extent of ROS-mediated toxicity due to pesticides exposure[13]. Moreover, Abdalla *et al.*[11] found that smoking and pesticides exposure could be responsible for increasing of oxidative stress that may lead to hyperlipidemia among pesticides sprayers.

The aim of this study was determination of the role of oxidative stress due to occupational exposure to pesticides on male reproductive hormones, especially among smokers and the antagonistic role of antioxidant enzymes.

MATERIALS AND METHODS

Subjects: Case-control study was conducted. The study included two groups; 51 agricultural workers occupationally exposed to pesticides for more than 5 years and 50 control subjects not occupationally exposed to pesticides and matched in age, special habits and socioeconomic status. Participants in this study were selected from village in Fayoum Governorate during the period August, 2016 to September, 2017.

Questionnaire: All enrolled participants fulfilled personal, environmental and occupational questionnaires during individual interview. Personal questionnaire included the age, gender and smoking habits. Environmental questionnaire was designed to exclude those workers who were exposed to huge amounts of pesticides from sources other than occupations and to exclude the controls that are exposed to huge amount of pesticides in their environment. Occupational questionnaire included type of the pesticides used mostly, the way of exposure, the duration of exposure in years and in days every year, use of personal protective equipment and safety measures and way of application.

Laboratory investigation: About 5 mL of the venous blood samples were collected from all the included workers and controls and were divided into three separate tubes. First tube was used for separation of serum to determinate levels of AChE, MDA, testosterone, LH and FSH. The second tube with EDTA, firstly to estimate GSH in whole blood and separate plasma for determination of BuChE and GST activities. The third tube was with EDTA for separating red blood cell to determinate levels of SOD and GPx activities. All samples were stored at -70°C until assessment of the biomarkers.

- **Biomarkers of exposure:** An immunoassay kit was used for *in vitro* quantitative determination of human acetylcholinesterase (AChE) concentration in the serum by (Sandwich-ELISA), Sunredbio, Shanghai (https://www.lsbio.com/elisakits/human-ache-acetylcholinesterase-elisa-kit-sandwich-elisa-ls-f9290/9290)
- The activity of butyrylcholinesterase (BuChE) was measured in plasma by colorimetric method according to Knedel and Bottger[14]
- **Biomarker of oxidative stress:** Serum MDA activity was determined by colorimetric method according to Ohkawa *et al.*[15]

- **Antioxidant biomarkers:** Glutathione reduced (GSH) concentration was determined in whole blood sample by colorimetric method according to Beutler *et al*.[16]
- Superoxide dismutase (SOD) activity was determined colorimetrically according to Nishikimi *et al*.[17]
- Glutathione-S-Transferase (GST) activity was determined in plasma by colorimetric method according to Habig *et al*.[18]
- Glutathione peroxidase (GPx) was determined using colorimetric method according to Paglia and Valentine[19]
- Male sex hormones
- Testosterone was estimated by Elisa kit[20]
- LH was estimated by ELISA kit[21]
- FSH was estimated by ELISA kit[22]

Statistical analysis: Statistical analysis of the collected data and the laboratory results was performed with SPSS version 18. The included workers and controls were divided according to their smoking habits into two subgroups for each (smoker and non-smoker workers and smoker and non-smoker controls). Kruskal-Wallis H was used in the comparisons between the four subgroups, Mann-Whitney U was used to compare between each two subgroups and correlation coefficient using Partial correlation was used in testing the association between different variables among the workers after controlling for smoking. The $p \leq 0.05$ was considered to be significant. Tabulation and figures were illustrated using excel program.

RESULTS

All the examined subjects in this study were males. There was no significant difference in the age between the workers and the controls. All smoker workers and their corresponding controls were no significant difference as shown in Table 1. Agricultural workers included in the present study were exposed to pesticides during their working days for more than 5 years. About 62% of them used to wear special clothes during their working day, but not proper personal protective equipment's (PPE).

In Table 2, AChE and BuChE in the smoker and non-smoker workers were significantly lower than both smokers and non-smoker controls (<0.05, <0.0001 respectively). The MDA and GPx concentrations were significantly increased in smoker and non-smoker workers compared to smoker and non-smoker controls (<0.005, <0.005, respectively). Moreover, MDA was significantly increased. The GST activities in smoker workers were significantly increased than in non-smoker controls and in non-smokers worker than in smokers and non-smokers controls (<0.001). The GSH concentrations in smoker and non-smoker workers were significantly lower than in smoker and non-smoker controls (<0.005). The SOD was also significantly decreased in smoker and non-smoker pesticide exposed workers compared to the non-smoker controls (<0.05).

Table 1: General information about agricultural pesticides exposed workers and unexposed controls

Parameters	Controls	Workers
Number of individuals	50.0	51.0
Mean age	33.4±7.5	35.4±11.8
Duration of exposure (year)	-	9.0± 3.5
Duration of exposure(day/year)	-	141.5±11.3
Smoking habit		
Smokers	16.0 (32%)	17.0 (35%)
Non-smokers	34.0 (68%)	33.0 (65%)

Table 2: Comparison of different estimated biochemical parameters between workers and controls

	Control (50)		Workers (51)		
Parameters	Smoker (16) (Mean±SD)	Non-smokers (34) (Mean±SD)	Smoker (18) (Mean±SD)	Non-smokers (33) (Mean±SD)	p-value*
Exposure biomarkers					
AChE (ng mL^{-1})	613.40±426.8	571.00±376.8	382.3±255.3[ab]	338.50±202.79[cd]	<0.05
BuChE (U L^{-1})	3595.00±1030.8	3739.18±1082.08	2503.1±614.2[ab]	2678.70±529.3[cd]	<0.0001
Oxidative stress biomarker					
MDA (nmol mL^{-1})	2.71±1.17	2.31±0.76	6.83±1.97[abf]	5.92±1.90[cdf]	<0.005
Antioxidant status					
GSH (mg dL^{-1})	38.50±15.8	38.10±9.25	24.9±7.6[a,b]	20.97±4.5[c,d]	<0.005
GST (U L^{-1})	375.40±120.7	291.00±106.4	438.5±163.4[b]	463.70±169.4[c,d]	<0.001
GPx (mU mL^{-1})	227.30±154.5	320.10±182.6	455.6±165.7[a,b]	489.70±173.0[c,d]	<0.005
SOD (U mL^{-1})	184.20±53.8	203.87±77.1	169.2±39.2[b]	154.70±50.3[d]	<0.05

* Significant difference was calculated using Kruskal-Wallis test, [a]Significant in comparison of smoker workers and smoker controls, [b]Significant in comparison of smoker workers and non-smoker controls, [c]Significant in comparison of non-smoker workers and smoker controls, [d]Significant in comparison of non-smoker workers and non-smoker controls, [f]Significant in comparison of smoker workers and non-smoker workers

Table 3: Comparison of male sex hormones between workers and controls

	Control		Workers		
Parameters	Smoker (16) (Mean±SD)	Non-smokers (34) (Mean±SD)	Smoker (18) (Mean±SD)	Non-smokers (33) (Mean±SD)	p-value*
FSH (mIU mL^{-1})	3.54±1.07	3.74±0.76	11.94±2.81^{a,b,f}	5.84±1.02^{f}	<0.01
LH (mIU mL^{-1})	10.58±7.58	6.55±4.08	12.32±5.79	9.91±4.66	0.294
Testosterone (ng mL^{-1})	2.88±1.88	2.67±1.99	3.25±4.12	3.43±4.08	0.855

* Significant difference was calculated using Kruskal-Wallis test, [a]Significant in comparison of smoker workers and smoker controls, [b]Significant in comparison of smoker workers and non-smoker controls, [f]Significant in comparison of smoker workers and non-smoker workers

Table 4: Correlation analyses between exposure biomarkers, oxidative stress and antioxidant biomarkers, in pesticides-exposed subjects

Parameters	AChE (ng mL^{-1})	BuChE (U L^{-1})	MDA (nmol mL^{-1})
AChE (ng mL^{-1})	-	0.3*	-0.3*
BuChE (U L^{-1})	0.3*	-	-0.5*
MDA (nmol mL^{-1})	-0.3*	-0.5*	-
GSH (mg dL^{-1})	0.2*	0.5*	-0.2
GST (U L^{-1})	-0.1	-0.2*	0.3*
GPx (mU mL^{-1})	-0.3*	-0.3*	0.1
SOD (IU L^{-1})	0.3*	0.3*	-0.1

* Correlation is significant at the 0.05 level (2-tailed)

Table 5: Correlation analyses between oxidative stress and antioxidant biomarkers and male sex hormones in pesticides-exposed subjects

Parameters	AChE (ng mL^{-1})	BuChE (U L^{-1})	MDA (nmol mL^{-1})	GSH (mg dL^{-1})	GST (U L^{-1})	GPx (mU mL^{-1})	SOD (IU L^{-1})
FSH (mIU mL^{-1})	-0.1	-0.2	-0.1	-0.2	0.1	-0.2	0.4*
LH (mIU mL^{-1})	-0.1	-0.1	-0.2	-0.1	-0.2	-0.1	0.3*
Testosterone (ng mL^{-1})	0.1	-0.2	-0.2	-0.1	0.2	-0.1	0.2

*Correlation is significant at the 0.05 level (2-tailed)

As Table 3 showed that there was significant increase in the FSH concentration in smoker workers compared to non-smoker workers and smoker and non-smoker controls (<0.01). But, LH concentration and testosterone showed non-significance elevation in the workers than their controls.

Data in Table 4 showed that there was significant correlation between AChE and BuChE (0.002) and significant negative correlations between MDA and the levels of AChE and BuChE in the exposed workers (0.004, <0.0001, respectively). While, the AChE and BuChE were significantly correlated with GSH (0.004, <0.0001, respectively) and with SOD (0.02, 0.005, respectively) and negatively correlated with GPx (0.003) and BuChE was also negatively correlated with GST (0.02) and MDA was significantly correlated with GST (0.05). As shown in Table 5, there were no significant correlations between hormones and the levels of AChE, BuChE and MDA. While, FSH and LH were significantly correlated with SOD (0.006, 0.01, respectively) but, testosterone showed no correlations with all the antioxidant enzymes.

DISCUSSION

Several pesticides have been considered as endocrine disruptors because of their capacity to block or activate hormone receptors including the effects on sex hormone levels [23]. Smoking could magnify those adverse effects due to the presence of large variety of compounds that could initiate or amplify oxidative damage[11]. Nordin *et al.*[24] mentioned that smoking during pesticide spraying can increase the absorption of pesticides both through inhalation of the airborne particles and through oral ingestion of the pesticide particles attached to cigarettes.

The results in the present study revealed that AChE and BuChE were significantly decreased among the examined workers compared to their controls due to their occupational exposure to pesticides and that both AChE and BuChE were significantly correlated with each other. A decline in AChE and BuChE is not attributed to their smoking habits, as the significant difference was in the smoker and non-smoker workers compared to smoker and non-smoker controls and no significant difference detected between smokers and non-smokers in both groups. This result was in agreement with Dahlan *et al.*[25], they mentioned that there was no association detected between smoking and AChE activities. The activity of AChE in human red blood cells (RBCs) may be considered as a diagnostic tool for risk assessment of toxicity due to pesticides exposure[26]. Also, the results of BuChE in the present study were in agreement with the results of Rastogi *et al.*[27]. The significant decrease in BuChE was more detectable than the significant decrease in AChE, this could be attributed to the sensitivity of BuChE as a biomarker of OP and CB exposure in humans. This noticeable result is detected also

by Araoud *et al.* [28] and Marsillach *et al.*[29], they attributed that to the short half-life of BuChE in the plasma compared to the AChE, which means that BuChE is a more sensitive indicator of recent pesticide exposure. Therefore, BuChE could be used as a biochemical marker for recent exposure to pesticides in surveillance for acute toxicity from pesticides.

Pesticides, as well as smoking, can induce oxidative stress by generating free radicals and altering the free radical scavenging antioxidant enzyme activity[30-32]. Oxidative stress biomarker MDA in the present study was significantly high in pesticides workers smokers and non-smokers compared to their controls and in the smoker workers than non-smoker workers. Moreover, significant inverse correlations were detected between MDA levels and both AChE and BuChE. Therefore, the elevation of MDA among the workers was attributed to their occupational exposure to pesticides and smoking was considered to have an additional elevating effect of MDA among the smoker workers.

Moreover, pesticides exposure may directly or indirectly modify the antioxidant defense capability of exposed subjects and thus increase their susceptibility to oxidative stress[33]. It is suggested that the activity of antioxidant enzymes may have an important mechanism in reducing the induction of pesticides chronic toxicities in occupationally exposed workers[34,35].

The present results revealed that GSH concentration were significantly decreased in smoker and non-smoker workers than smoker and non-smoker controls. The same results were detected by Sharma *et al.*[36]. Moreover, GSH was found to be correlated significantly with AChE. This non-enzymatic antioxidant has important role in binding with the free radicals.

Tillman *et al.*[37] found significant reduction in GST in insecticides exposed workers in comparison to the control group. Ranjbar *et al.*[38] explained the decrease in GST activity to its antagonistic direct binding effect to the free radicals attributed to pesticide exposure. But, our result revealed that GST activities was significantly increased in non-smoker workers than both smoker and non-smoker controls, while, GST in smoker workers was significantly increased only than in the non-smoker controls and was inversely correlated with BuChE. The significant increase in GST activities in non-smokers workers could be attributed to the oxidative stress result from exposure to pesticides. But in the smoker workers, GST activities could be against oxidative stress produced from combined exposure to pesticides and smoking. This will explain that there was no significant difference in GST between smoker workers and smoker controls and significant correlation with MDA. This agrees with Thirumalai *et al.*[39], they suggested that the increased GST activities among smokers might be due to increase oxidative stress due to cigarette smoke exposure.

In the present study, the concentration of GPx was significantly increased in the workers (smokers and non-smokers) compared to their controls (smokers and non-smokers). These results are in agreement with Ogut *et al.*[40], they found that GPx was significantly increased in pesticides sprayers when compared to controls. Increased activity of GPx could be due to its antagonistic effect against the significant production of H_2O_2 in pesticide exposed workers that could lead to induction of the chronic toxicity [41]. The present results detected no significant difference in the GPx between smoker and non-smoker workers, while, it was significantly inversely correlated with AChE and BuChE. This revealed that smoking habit may have no synergistic effect on GPx production in the examined workers. This is against the Abdalla *et al.*[11], they found a synergistic effect of smoking with the occupational exposure to pesticides on GPx activates, as they observed significant increase in GPx in smoker pesticide sprayers compared to the non-smoker sprayers.

There is a debate about the defense action of SOD, as it is considered as one of the protective antioxidant against the superoxide anions (O^{2-}) by converting them into hydrogen peroxide (H_2O_2). In the present study, SOD activity was significantly decreased in smoker and non-smoker pesticide exposed workers compared to the non-smoker controls. This agrees with the results of Noshy *et al.*[12] and Lopez *et al.*[30], they found that SOD in the pesticides exposed subjects was significantly lower than their controls. Vidyasagar *et al.*[42] found that SOD levels increased with the increase in the severity of pesticides poisoning. Moreover, it was noticed in the current results, that inhibition of SOD could be due to the combined effects of exposure to both pesticides and smoking, as there was no significant difference in the SOD levels between the workers and the smoker controls and SOD was significantly correlated with AChE. This agrees with Lopez *et al.*[30], they found positive correlation between SOD and AChE activities in the workers with high exposure period to pesticides. Pasupathi *et al.*[43] detected the same results, as there was significant decrease in SOD activity in smokers than in non-smokers. The Quinone/hydroquinone radicals from cigarette smoke have the capability of reducing oxygen to superoxide radicals that inactivates SOD enzyme. While, Abdalla *et al.*[11] found that there is no significant effect of smoking on the activities of SOD and the effect was mainly due to exposure to pesticides, as SOD was significantly inversely correlated with the duration of exposure to pesticides.

Therefore, the present study noticed that oxidative stress was due to exposure to pesticides, in addition to the significant effect of smoking. This could be attributed to their wrong habits inform of smoking and having meals during working while applying pesticides. The same habits were detected in several studies[44-46]. This habits increase the risk from occupational exposure to pesticides.

Several studies denoted that pesticides could cause significant decreases of the reproductive male hormones; testosterone, LH and FSH[47-49]. Joshi and Sharma[49] attributed their results to the reduction in AChE activity due to pesticides and the blockage of nerve impulses, that may alter the release of pituitary hormones, namely FSH and LH. But in the present study, even there was significant reduction in AChE, there was significant elevation in FSH among the smoker workers compared to non-smoker workers and the controls (smoker and non-smoker). This could mean that smoking in combination with pesticides exposure had an adverse effect on FSH. Current results were partially agreed with the findings of Miranda-Contreras *et al.*[50]. They mentioned that there was a tendency for the increase FSH levels in pesticides exposed population, but they added that LH was also increased. The concentration of LH was not significantly affected in the present study, as there was no significant different between the workers and their controls; whether smokers or none. Also, the included sex hormones had no significant relation with the levels of AChE and BuChE.

Halmenschlager *et al.*[51] found that there was no association between smoking and male reproductive hormones, as there is no association between the increased pack-years of smoking and increased odds ratio for occurrence of low FSH, LH and testosterone. While, Svartberg and Jorde[52] found that testosterone was significantly correlated with the number of cigarettes smoked daily. It was found also that pesticides inhibit the non-specific esterase activity in Leydig cells that result in reduced testosterone production[53]. But present results revealed that testosterone was not significantly affected by occupational exposure to pesticides or smoking. Therefore, this study revealed that the combined exposure to pesticides and smoking may have no significant effect on LH and testosterone concentrations. This is in agreement with several studies[54-56], they reported that levels of total testosterone unchanged in male smokers and disagree with other studies which have reported that smoking may stimulate release of testosterone production not only by chronic mechanisms but also by acute one[57,58].

The present results revealed that the included male reproductive hormones; LH and testosterone were not significantly correlated with MDA, while, FSH was significantly correlated with it. The FSH and LH were found to be significantly correlated with SOD, but testosterone did not correlate with any of the antioxidant enzymes.

CONCLUSION AND RECOMMENDATIONS

From that it was concluded that, occupational exposure to pesticides could lead to significant oxidative stress. Smoking has a synergistic oxidative stress effect with the occupational exposure to pesticides on FSH. It seemed that SOD and GST play important roles in antagonizing this oxidative stress among the examined pesticides workers.

This study discovered the synergistic oxidative stress of exposure to smoking and pesticides, that can be beneficial for reduction of the oxidative stress due to occupational exposure to pesticides through cessation of smoking of the exposed workers. This study will help the researcher to uncover the critical areas of the significant effect of occupational exposure to pesticides on male reproductive hormones without significant correlation with the duration of occupational exposure that many researchers were not able to explore in the previous study. Thus a new theory on the synergistic oxidative stress of smoking and exposure to pesticides may be arrived at.

SIGNIFICANCE STATEMENT

This study showed that pesticides are highly toxic to living organisms they represent a major concern for human health and contribute significantly to many diseases and other chronic health effects. The study contributes to the effective monitoring not of agricultural environment only, but of all the public health and raising awareness on the hazards of using pesticides to human health and other living organisms.

ACKNOWLEDGMENT

Authors express appreciation to all persons who included in this study for helping us in completing this work.

REFERENCES

1. US-EPA., 2001. Diazinon revised risk assessment and agreement with registrants. Office of Pesticide Programs, US Environmental Protection Agency, USA.
2. Agrawal, A. and B. Sharma, 2010. Pesticides induced oxidative stress in mammalian systems: A review. Int. J. Biol. Med. Res., 1: 90-104.
3. Azmi, M.A., S.N.H. Naqvi, M.A. Azmi and M. Aslam, 2006. Effect of pesticide residues on health and different enzyme levels in the blood of farm workers from Gadap (rural area) Karachi-Pakistan. Chemosphere, 64: 1739-1744.

4. Remor, A.P., C.C. Totti, D.A. Moreira, G.P. Dutra, V.D. Heuser and J.M. Boeira, 2009. Occupational exposure of farm workers to pesticides: Biochemical parameters and evaluation of genotoxicity. Environ. Int., 35: 273-278.
5. Bjorling-Poulsen, M., H.R. Andersen and P. Grandjean, 2008. Potential developmental neurotoxicity of pesticides used in Europe. Environ. Health, Vol. 7. 10.1186/1476-069X-7-50.
6. Sarkar, R., K.P. Mohanakumar and M. Chowdhury, 2000. Effects of an organophosphate pesticide, quinalphos, on the hypothalamo-pituitary-gonadal axis in adult male rats. J. Reprod. Fertil., 118: 29-38.
7. Kang, H.G., S.H. Jeong, J.H. Cho, D.G. Kim, J.M. Park and M.H. Cho, 2004. Chlropyrifos-methyl shows anti-androgenic activity without estrogenic activity in rats. Toxicology, 199: 219-230.
8. Meeker, J.D., L. Ryan, D.B. Barr and R. Hauser, 2006. Exposure to nonpersistent insecticides and male reproductive hormones. Epidemiology, 17: 61-68.
9. Blanco-Munoz, J., M.M. Morales, M. Lacasana, C. Aguilar-Garduno, S. Bassol and M.E. Cebrian, 2010. Exposure to organophosphate pesticides and male hormone profile in floriculturist of the state of Morelos, Mexico. Hum. Reprod., 25: 1787-1795.
10. Slimani, S., M.S. Boulakoud and C. Abdennour, 2011. Pesticide exposure and reproductive biomarkers among male farmers from North-East Algeria. Ann. Biol. Res., 2: 290-297.
11. Abdalla, M.S., A. Saad-Hussein, K.S. Ibrahim, H.A. El-mezayen and N.F. Osman, 2015. Effects of smoking on the oxidant/antioxidant balance and the blood lipids in pesticide sprayers. Toxicol. Ind. Health, 31: 173-178.
12. Noshy, M.M., A. Saad-Hussein, E.M. Shahy, H.M. El-Shorbagy, M.M. Taha and E.A. Abdel-Shafy, 2017. Assessment of anticholinesterase toxicity, oxidative stress and antioxidant status in carbamate and organophosphorus pesticides-exposed agricultural workers. Int. J. Pharmaceut. Clin. Res., 9: 205-209.
13. Singh, M., R. Sandhir and R. Kiran, 2006. Erythrocyte antioxidant enzymes in toxicological evaluation of commonly used organophosphate pesticides. Indian J. Exp. Biol., 44: 580-583.
14. Knedel, M. and R. Bottger, 1967. [A kinetic method for determination of the activity of pseudocholinesterase (acylcholine acyl-hydrolase 3.1.1.8.)]. Klin. Wochenschr. J., 45: 325-327.
15. Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.
16. Beutler, E., O. Duron and B.M. Kelly, 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882-888.
17. Nishikimi, M., N.A. Rao and K. Yagi, 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun., 46: 849-854.
18. Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.
19. Paglia, D.E. and W.N. Valentine, 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med., 70: 158-169.
20. Chen, A., J.J. Bookstein and D.R. Meldrum, 1991. Diagnosis of a testosterone-secreting adrenal adenoma by selective venous catheterization. Fertil. Steril., 55: 1202-1203.
21. Knobiol, E., 1980. The neuroendocrine control of the menstrual cycle. Recent Prog. Hormone Res., 36: 53-88.
22. Uotila, M., E. Ruoslahti and E. Engvall, 1981. Two-site sandwich enzyme immunoassay with monoclonal antibodies to human alpha-fetoprotein. J. Immunol. Methods, 42: 11-15.
23. Vinggaard, A.M., C. Hnida, V. Breinholt and J.C. Larsen, 2000. Screening of selected pesticides for inhibition of CYP19 aromatase activity *in vitro*. J. Toxico. *In Vitro*, 14: 227-234.
24. Nordin, R.B., S. Araki, H. Sato, K. Yokoyama, W.A.M.B.W. Muda and D.W. Kyi, 2002. Effects of safety behaviours with pesticide use on occurrence of acute symptoms in male and female tobacco-growing Malaysian farmers. Ind. Health, 40: 182-190.
25. Dahlan, M., T. Suwandi and L. Dewanti, 2015. Analysis of factors related cholinesterase activities to the family farmers in Pattapang sub district of Malino. Int. J. Technol. Enhancements Emerg. Eng. Res., 3: 41-43.
26. Maroni, M., C. Colosio, A. Ferioli and A. Fait, 2000. Biological monitoring of pesticide exposure: A review. Introduction. Toxicology, 143: 1-118.
27. Rastogi, S.K., P.V.V. Satyanaraynan, D. Ravishankar and S. Tripathi, 2009. A study on oxidative stress and antioxidant status of agricultural workers exposed to organophosphorus insecticides during spraying. Ind. J. Occup. Health., 13: 131-134.
28. Araoud, M., F. Neffeti, W. Douki, H.B. Hfaiedh, M. Akrout, M.F. Najjar and A. Kenani, 2011. Factors influencing plasma butyrylcholinesterase activity in agricultural workers. Ann. Biol. Clin., 69: 159-166.
29. Marsillach, J., R.J. Richter, J.H. Kim, R.C. Stevens and M.J. MacCoss *et al*., 2011. Biomarkers of organophosphorus (OP) exposures in humans. Neurotoxicology, 32: 656-660.
30. Lopez, O., A.F. Hernandez, L. Rodrigo, F. Gil and G. Pena *et al*., 2007. Changes in antioxidant enzymes in humans with long-term exposure to pesticides. Toxicol. Lett., 171: 146-153.
31. Mecdad, A.A., M.H. Ahmed, M.E. El Halwagy and M.M. Afify, 2011. A study on oxidative stress biomarkers and immunomodulatory effects of pesticides in pesticide-sprayers. Egypt. J. Forensic Sci., 1: 93-98.
32. Abdul-Rasheed, O.F. and W.T. Al-Rubayee, 2013. Effects of cigarette smoking on lipid peroxidation and antioxidant status in Iraqi men at Baghdad city. Int. J. Basic Applied Sci., 2: 47-50.
33. Rai, D.K., P.K. Rai, S.I. Rizvi, G. Watal and B. Sharma, 2009. Carbofuran-induced toxicity in rats: Protective role of vitamin C. Exp. Toxicol. Pathol., 61: 531-535.

34. Bhanti M. and A. Taneja, 2007. Contamination of vegetables of different seasons with organophosphorous pesticides and related health risk assessment in northern India. Chemosphere, 69: 63-68.
35. Muniz, J.F., L. McCauley, J. Scherer, M. Lasarev and M. Koshy *et al*., 2008. Biomarkers of oxidative stress and DNA damage in agricultural workers: A pilot study. Toxicol. Applied Pharmacol., 227: 97-107.
36. Sharma, R.K., G. Upadhyay, N.J. Siddiqi and B. Sharma, 2013. Pesticides-induced biochemical alterations in occupational North Indian suburban population. Hum. Exp. Toxicol., 32: 1213-1227.
37. Tillman, R.W., M.R. Siegel and J.W. Long, 1973. Mechanism of action and fate of the fungicide chlorothalonil (2, 4, 5, 6-tetrachloroisophthalonitrile) in biological systems: I. Reactions with cells and subcellular components of *Saccharomyces pastorianus*. Pesticide Biochem. Physiol., 3: 160-167.
38. Ranjbar, A., H. Solhi, F.J. Mashayekhi, A. Susanabdi, A. Rezaie and M. Abdollahi, 2005. Oxidative stress in acute human poisoning with organophosphorus insecticides; a case control study. Environ. Toxicol. Pharmacol., 20: 88-91.
39. Thirumalai, T., S.V. Therasa, E.K. Elumalai and E. David, 2010. Effect *in vivo* of cigarette smoke on lipid peroxidation and antioxidant status in male *albino* mice. J. Pharm. Sci. Res., 2: 579-582.
40. Ogut, S., F. Gultekin, A.N. Kisioglu and E. Kucukoner, 2011. Oxidative stress in the blood of farm workers following intensive pesticide exposure. Toxicol. Ind. Health, 27: 820-825.
41. Singh, N., V.K. Gupta, A. Kumar and B. Sharma, 2017. Synergistic effects of heavy metals and pesticides in living systems. Front. Chem., Vol. 5. 10.3389/fchem.2017.00070.
42. Vidyasagar, J., N. Karunakar, M.S. Reddy, K. Rajnarayana, T. Surender and D.R. Krishna, 2004. Oxidative stress and antioxidant status in acute organophosphorous insecticide poisoning. Ind. J. Pharmacol., 36: 76-79.
43. Pasupathi, P., G. Saravanan and J. Farook, 2009. Oxidative stress bio markers and antioxidant status in cigarette smokers compared to nonsmokers. J. Pharm. Sci. Res., 1: 55-62.
44. Mekonnen, Y. and T. Agonafir, 2002. Pesticide sprayers' knowledge, attitude and practice of pesticide use on agricultural farms of Ethiopia. Occup. Med., 25: 311-315.
45. Salameh, P.R., I. Baldi, P. Brochard and B.A. Saleh, 2004. Pesticides in Lebanon: A knowledge, attitude and practice study. Environ. Res., 94: 1-6.
46. Recena, M.C.P., E.D. Caldas, D.X. Pires and E.R.J. Pontes, 2006. Pesticides exposure in culturama, Brazil-knowledge, attitudes and practices. Environ. Res., 102: 230-236.
47. Lotti, F., G. Corona, P. Vitale, E. Maseroli, M. Rossi, M.G. Fino and M. Maggi, 2015. Current smoking is associated with lower seminal vesicles and ejaculate volume, despite higher testosterone levels, in male subjects of infertile couples. Hum. Reprod., 30: 590-602.
48. Richthoff, J., S. Elzanaty, L. Rylander, L. Hagmar and A. Giwercman, 2008. Association between tobacco exposure and reproductive parameters in adolescent males. Int. J. Androl., 31: 31-39.
49. Joshi, S.C. and P. Sharma, 2012. Effect of acephate on sex hormones, sperm dynamics and fertility in male albino rats. Int. J. Res. Pharm. Biomed. Sci., 3: 286-292.
50. Miranda-Contreras, L., R. Gomez-Perez, G. Rojas, I. Cruz and L. Berrueta *et al*., 2013. Occupational exposure to organophosphate and carbamate pesticides affects sperm chromatin integrity and reproductive hormone levels among Venezuelan farm workers. J. Occup. Health, 55: 195-203.
51. Halmenschlager, G., S. Rossetto, G.M. Lara and E.L. Rhoden, 2009. Evaluation of the effects of cigarette smoking on testosterone levels in adult men. J. Sexual Med., 6: 1763-1772.
52. Svartberg, J. and R. Jorde, 2007. Endogenous testosterone levels and smoking in men. The fifth Tromso study. Int. J. Androl., 30: 137-143.
53. Abou El-Magd, S.A., L.M. Sabik and A. Shoukry, 2011. Pyrethroid toxic effects on some hormonal profile and biochemical markers among workers in pyrethroid insecticides company. Life Sci. J., 8: 311-322.
54. Recio, R., G. Ocampo-Gomez, J. Moran-Martínez, V. Borja-Aburto and M. Lopez-Cervantes *et al*., 2005. Pesticide exposure alters follicle-stimulating hormone levels in Mexican agricultural workers. Environ. Health Perspect., 113: 1160-1163.
55. Kapoor, D. and T.H. Jones, 2005. Smoking and hormones in health and endocrine disorders. Eur. J. Endocrinol., 152: 491-499.
56. Mendelson, J.H., M.B. Sholar, N.H. Mutschler, M. Jaszyna-Gasior, N.V. Goletiani, A.J. Siegel and N.K. Mello, 2003. Effects of intravenous cocaine and cigarette smoking on luteinizing hormone, testosterone and prolactin in men. J. Pharmacol. Exp. Ther., 307: 339-348.
57. Bretveld, R., M. Brouwers, I. Ebisch and N. Roeleveld, 2007. Influence of pesticides on male fertility. Scand. J. Work Environ. Health, 33: 13-28.
58. Isidori, A.M., E. Giannetta, D. Gianfrilli, E.A. Greco and V. Bonifacio *et al*., 2005. Effects of testosterone on sexual function in men: Results of a meta-analysis. Clin. Endocrinol., 63: 381-394.