Assessment of Serum Malondialdehyde among Rural Punjabi Pesticide Sprayers

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Abstract

The study was designed to evaluate chronic effects of cocktail of pesticides on serum Malondialdehyde in rural Punjabi spray workers. These occupational sprayers are continuously exposed to a mixture of pesticides. The impact of the pesticides may be detected by ensuring biochemical changes even before adverse clinical health effects can occur. The sprayers were divided into various categories on the basis of pesticides used i.e. exposure time, total exposure period, age and body mass index (BMI). The mean±S.D. activity of MDA in control group was found to be 6.24±3.32 nmol/ml. The data generated was statistically analyzed by applying student's '*t* ' test and one -way analysis of variance (ANOVA). A significant elevation in MDA activity was observed in all categories of spray workers.

KEYWORDS: Pesticides, Spray Workers, Malondialdehyde.

INTRODUCTION

Pesticides constitute a heterogeneous category of chemicals specifically designed for the control of pests (Bhalli *et al.,* 2006) and they have significant economic, environmental and public health impacts. Their application helps to improve human nutrition through greater availability, longer shelf life and lower cost of food (Elhalwagy and Zaki, 2008). Presence of pesticides in the ecosystem leads to development of various types of morphological, physiological, biochemical and behavioral changes in individuals (Kamsin, 1997 and Patil *et al*., 2003).

In India, 15-20% of the total harvest is destroyed by pests resulting in uncontrolled use of pesticides by Indian cultivators (Khan, 2012). The condition is quite grim in Punjab as the farmers spray 5 to 6 times more pesticides than recommended by Punjab Agricultural University (Mathur *et al*., 2005).

The mode of action of most of the pesticides is induction of oxidative stress leading to generation of free radicals and alterations in antioxidant or oxygen free radical scavenging system. The stimulation of free radical production, induction of lipid peroxidation and disturbance of the total antioxidant capability of the body, are toxicity mechanisms of most pesticides, including organophosphates, bipyridyl herbicides, and organochlorine (Abdollahi *et al.,* 2004). The toxic effects are caused due to the production of peroxide and free radicals that damage all the components of the cell, including protein, lipids, DNA and RNA (Muniz *et al.,* 2008). Reactive oxygen species (ROS) arise as byproduct of normal cellular metabolism or may be consequence of exposure to certain chemicals (Kerr *et al*., 1996; Krieger and Caruso, 2001). A significant elevated malondialdehyde (MDA) (end product of lipid peroxidation) level has been observed in the sprayers exposed to organophosphate, carbamate and organochlorine pesticides when compared to the controls (Prakasam *et al.,* 2001b), suggesting that

oxidative stress may be involved in the toxicity of pesticides (Hai *et al.,* 1997; Bachowski *et al.,* 1998). Moreover higher oxidative stress in pesticide sprayers is evidenced by increased concentration of plasma and red cell thiobarbituric acid reactive substances (TBARS), changes in antioxidant status, and altered activities of cellular enzymes (Prakasam *et al.,* 2001b).

This research work has been designed to study the effect of cocktail of pesticides on Serum Malondialdehyde concentration in rural sprayers of District Bathinda, Punjab.

MATERIAL AND METHODS

A total of 283 subjects from Bathinda district of Punjab Province were randomly selected for this study. Out of these 183 subjects were spray workers who were exposed since their childhood to a mixture of pesticides and 100 controls who had no history of exposure to chemicals or other genotoxic substances were also selected from same geographical area. These spray workers were being exposed to a mixture of various types of pesticides during pesticide spraying season on different crops. Most of the spray work was done without using any protective measures like caps, gloves, full sleeve shirts, face masks and shoes etc.

The sprayers were divided into various categories on the basis of pesticides exposure time, total exposure period, age and body mass index (BMI). To observe the activity of Serum Malondialdehyde, these categories were further divided into following subcategories (i) ≤5 years of exposure, (ii) 5-10 years exposure and (iii) ≥10 years of exposure; (a) during spray, (b) within 1-10days of spraying, (c) 10-25days and (d) ≥25 days of spraying; Age (1) ≤25year, (2) 25-35year, (3) 35-45year and (4) 45-60 years; and BMI (A) ≤18.5, (B) 18.6-25 and (C) 25.1-30. The most commonly used pesticides by farmers were (i) Monocrotophos, (ii) Chlorpyrifos, (iii) Profenofos, (iv) Acephate, (v) DDVP, (vi) Endosulfan, (vii) Rift, (viii) Confidor, (ix) Acetamiprid, (x) Round up, (xi) Matador, (xii) Topic and (xiii) Leader.

All the participants signed a written consent and answered a standard questionnaire regarding the demographic (age, gender etc.) as well as the questions pertaining to medical history (any particular disease, vaccination, medication etc), smoking habit and occupational exposure (years of exposure). The sprayers were also informed about the purpose of the study and the data was collected with their consent.

Blood samples (5ml) were collected from cubital vein of all the participants with the help of a trained medical technician using sterile disposable syringe. Blood was left as such in syringe. The samples were kept in an ice box and were brought to the laboratory for making serum by centrifugation. Serum was made by centrifuging at 2000 to 3000 rpm for 15 to 20 minutes and was kept in well cleaned and labeled plastic vials for enzymatic analysis.

Malondialdehyde was used as a biochemical marker for lipid peroxidation and was estimated by the method of Satoh (1978) using Thiobarbituric acid. MDA was measure by reading the absorbance at 530nm and expressed as nmol/ml.

The data obtained was finally analyzed statistically using student t-test and analysis of variance (ANOVA). Values less than $p<0.05$ were considered significant. The present research work has been carried out according to the guidelines issued by the Institutional human ethical committee.

RESULTS AND DISCUSSION

The mean±S.D. activity of MDA in control group and various sprayers exposed to different pesticides shown in Table-I. It was found to be 6.24±3.32 nmol/ml for control group. The activities of MDA were found to be 09.92±07.18 nmol/ml; 12.91±05.69 nmol/ml; 11.10±06.64nmol/ml; 12.61±05.34nmol/ml; 18.16±12.18nmol/ml and 23.19±13.48nmol/ml in categories i, iii, viii, ix, xii and xiii of pesticides respectively and the observed elevation was significant ($p<0.0001$). While in categories (ii, v, vi and vii) the activities of MDA was found to be non significant ($p>0.05$) in comparison to control group.

The ANOVA values of MDA for exposed categories and unexposed population is given in Table-II which were again found to be significant ($p<0.0001$).

To evaluate the effects of pesticides according to exposure time, the mean value of control was compared with mean values of pesticide exposed categories (i, ii and iii). The mean values of MDA in pesticide exposed categories showed significant increase ($p<0.0001$) in all categories (10.26±7.38 nmol/ml; 9.11±6.48 nmol/ml and 9.56±6.71 nmol/ml) in comparison to control group (Fig. 1). Table-III shows the values of MDA of spray workers according to exposure time and control which was also found to be significant ($p<0.0001$).

The MDA concentration between various subcategories showed significant increase at ($p<0.0001$) in sprayers falling in categories (a) (10.38±7.28 nmol/ml) and (b) (10.44±7.61nmol/ml) and significant increase at ($p<0.01$) in category (c) (8.34±4.50 nmol/ml) when compared with control group. No significant change ($p>0.05$) was observed in category (d) (7.62±4.78 nmol/ml) (Fig. 2). The results of MDA for exposed categories and unexposed population is given in Table-IV which was again found to be significant ($p<0.0001$).

Significant increase ($p<0.0001$) in MDA concentration was found in all exposed categories (1) 10.39±7.24 nmol/ml; (2) 9.41±8.43 nmol/ml; (3) 9.23±4.35 nmol/ml and (4) 8.63±4.81 nmol/ml in comparison to control group (Fig. 3). As shown in Table-V significant variations ($p<0.0001$) in concentration of MDA in all categories of spray workers and control was observed.

The MDA concentration showed significant increase ($p<0.0001$) in sprayers in categories (B) (9.17±6.24 nmol/ml) and (C) (12.01±9.01 nmol/ml) and significant increase at $p<0.01$ in category (A, 9.41±6.88 nmol/ml) when compared with control group (Fig. 4). The values of MDA for exposed categories and unexposed population is given in Table-VI which were again found to be significant ($p<0.0001$).

In the present study cumulative effects of various pesticides was studied in professional spray workers. The MDA concentration was observed in younger sprayers as well as in those sprayers having longer exposure to pesticides. This may be attributed to the higher metabolic rate in young people and due to repeated exposures to pesticides in sprayers having longer exposure experience. According to Otitoju *et al.* (2008) lipid peroxidation depends on a lot of factors e.g. age, sex and concentration of xenobiotics. Younger people especially children exposed to insecticide contaminated diet or environment are at higher health risk as their internal organs and tissues are still developing. They also have higher feeding metabolic rates, hence, generate more free radicals which may attack or damage cell membranes, affecting membrane integrity and membrane dependent functions.

MDA production in the present research was found to be statistically significant ($p<0.05$) in each category which may be due to decreased activity of antioxidant enzymes in the presence of mixture of pesticides which results in increased peroxidation of membranes. This is also supported by the work of Simoniello (2010) who made a study on rural sprayers of Argentina.

Different organophosphates such as phosalane, ethyl, chlorpyrifos and diazinon are shown to induce oxidative stress as shown by enhanced MDA production (Prakasam *et al.,* 2001a; Altuntas *et al.,* 2003; Catagol *et al.,* 2007). Similarly carbamates and some pyrethroids are also shown to generate free radicals and variation in antioxidant enzymes (Prasanthi *et al.,* 2005; Dettbarn *et al.,* 2006; El-Demerdash, 2007).

Several different mechanisms have been proposed for generation of free radicals leading to lipid peroxidation. The normal cellular function depends on a balance between reactive oxygen species produced and antioxidant defense mechanisms available to the cell. ROS arise as by-products of normal cellular metabolism or may be the consequence of exposure to certain chemicals (Moslen, 1994; Kerr *et al.,* 1996; Krieger and Caruso, 2001). According to Kappus (1987); Comporti (1989); Fernandez *et al.* (2003) ROS derived from chemicals, oxygen or nitrogen have been implicated as putative noxious intermediates responsible for cellular damage. Because electrophlic metabolites or radicals and excited species can readily interact with essential bimolecules, covalent bonding to cellular components or their oxidative modification can occur, leading to structural and functional alterations.

Similarly Oberley (2002) observed that ROS are generated in mitochondria and other sub cellular locations of normal mammalian cells, as a by-product of normal respiration or as a function of biochemical reaction using oxygen. Also, the cell has an intricately regulated antioxidant defense system to protect against toxic effects of ROS and to modulate physiological effects of ROS.

Various researchers (Griveau *et al.,* 1995; Gill *et al.,* 2002; Singh *et al.,* 2007) suggested that significant increase in peroxidation of membrane lipids can be due to production of free radicals, which occurs when $\dot{O}H$ radical produced in close vicinity to membrane and attacks the fatty acid side chains of membrane phospholipids preferentially poly-unsaturated fatty acid chains, which further undergoes molecular rearrangements to produce conjugated dienes, structure that can have various fates i.e. such two radicals can combine to form a covalent bond or they can also react with proteins. However under physiological conditions the most likely fate of carbon centered radicals is to combine with oxygen to create yet another radical that is peroxyl radical. Peroxyl radicals are reactive enough to attack adjacent fatty acid side chains, abstracting hydrogen to produce yet another carbon central radical and so the chain reaction continues. One $\dot{O}H$ radical can result in the conversion of many hundred fatty acid side chains into lipid peroxides. A significant increase in lipid peroxidation in pesticides exposed worker might lead to susceptibility of the bio membrane and cause tissue injury.

Wang (2004) and Saxena *et al.* (2011) hypothesized that pesticides induce oxidative stress by inhibiting the mitochondrial electron transfer chain reaction leading to accumulation of semi ubiquitous, which enables it to transfer one electron ($\bar{e}$) to molecular oxygen to form superoxide radicals.

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Table-I Comparison of Malondialdehyde (MDA) according to Categories of pesticides sprayed by Spray Workers with control

			MDA			%age change	t value Mean
			Mean	S.D	S.E.M		
Control (N=100)			06.24	03.32	00.32		
Spray workers (N=183)	Organophosphate	(i) Monocrotophos (N=48)	09.92	07.18	01.03	58.97	4.31^{***}
		(ii) Chlorpyrifos (N=17)	06.04	03.02	00.73	-03.21	$0.81^{N.S}$
		(iii) Profenofos (N=15)	12.91	05.69	01.47	106.89	6.62^{***}
		(iv) Acephate (N=11)	09.75	05.37	01.62	56.25	3.16^{**}
		(v) DDVP (N=09)	06.40	04.26	01.42	02.56	$0.13^{N.S}$
	Organochlorine	(vi) Endosulfan (N=05)	08.24	04.23	01.89	32.05	$1.36^{N.S}$
		(vii) Rift (N=04)	06.81	03.47	01.73	9.13	$0.34^{N.S}$
	Neonicotinoid	(viii) Confidor (N=74)	11.10	06.64	00.77	77.88	6.37^{***}
		(ix) Acetamipride powder (N=20)	12.61	05.34	01.91	102.08	7.11^{***}
	Phosphanoglycine	(x) Round up (N=04)	10.97	04.77	02.38	75.80	2.81^{**}
	Pyrethroid	(xi) Matador (N=04)	02.44	00.78	00.39	-60.89	2.02^{*}

	---------	(xii) Topic (N=17)	18.16	12.18	02.95	191.02	8.34***
	---------	(xiii) Leader (N=04)	23.19	13.48	06.74	271.63	8.44***

*Significant at p<0.05 S.D= Standard deviation 't'= Student's t test
** Significant at p<0.01 S.E.M= Standard error mean
*** Significant at p<0.0001 N.S= Non significant

Table-II Analysis of variance for Malondialdehyde (MDA) according to Categories of pesticides sprayed by Spray Workers with control

Source of Variation	SS	df	MS	F
Between Groups	4164.421	13	320.3401	8.96***
Within Groups	11365.13	318	35.7394	
Total	15529.55	331		

SS= Sum of Squares ***Significant at p<0.0001 F= One way ANOVA test MS= Mean of squares df= Degree of freedom

Table-III Analysis of variance for Malondialdehyde (MDA) according to pesticide exposure time of Spray Workers with control

Source of Variation	**SS**	**df**	**MS**	**F**
Between Groups	742.3131	3	247.4377	7.42***
Within Groups	9293.442	279	33.30983	
Total	10035.75	282		

*** Significant at p<0.0001 SS= Sum of Squares df= Degree of freedom MS= Mean of squares
F= One way ANOVA test

Table-IV Analysis of variance for Malondialdehyde (MDA) according to pesticide Spray time of Spray Workers with control

Source of Variation	**SS**	**df**	**MS**	**F**
Between Groups	1747.973	4	436.9934	9.91***
Within Groups	12258.17	278	44.09414	
Total	14006.14	282		

*** Significant at p<0.0001 SS= Sum of Squares df= Degree of freedom MS= Mean of squares
F= One way ANOVA test

Table-V Analysis of variance for Malondialdehyde (MDA) according to age of Spray Workers with control

Source of Variation	SS	df	MS	F
Between Groups	1734.688	4	433.672	9.34^{***}
Within Groups	12901.01	278	46.4065	
Total	14635.69	282		

*** Significant at p<0.0001 SS= Sum of Squares df= Degree of freedom
MS= Mean of squares
F= One way ANOVA test

Table-VI Analysis of variance for Malondialdehyde (MDA) according to Body Mass Index (BMI) of Spray Workers with control

Source of Variation	SS	df	MS	F
Between Groups	882.7794	3	294.2598	8.97^{***}
Within Groups	9151.366	279	32.80059	
Total	10034.15	282		

*** Significant at p<0.0001 SS= Sum of Squares df= Degree of freedom
MS= Mean of squares
F= One way ANOVA test

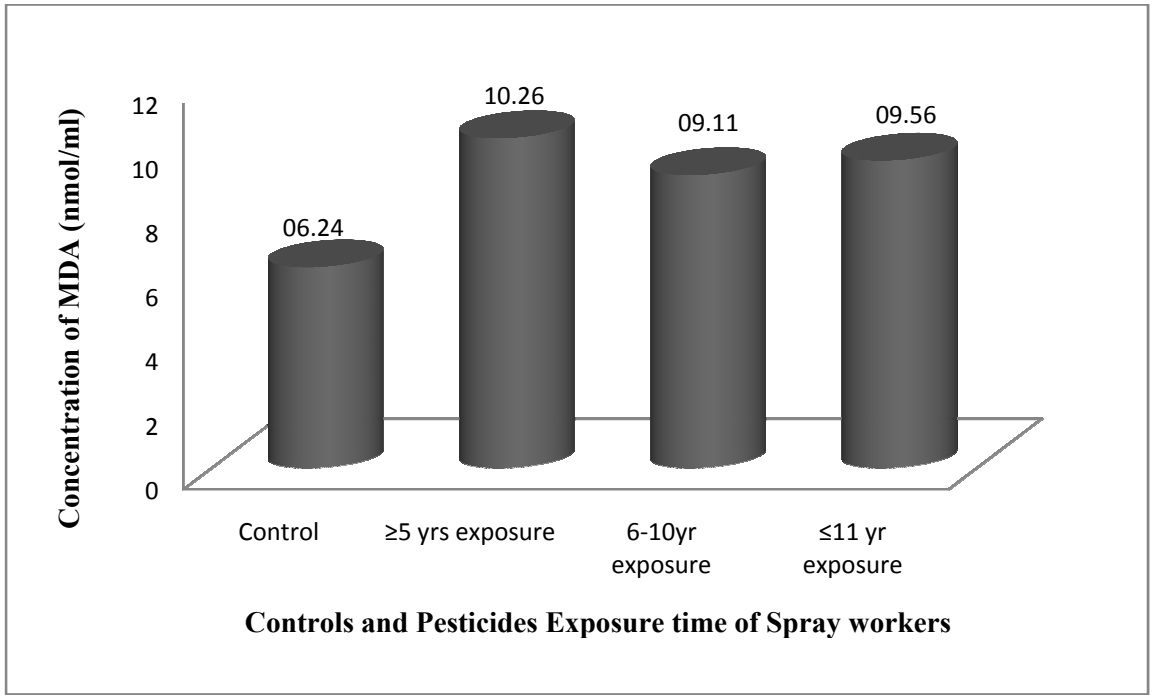

Fig. 1 Comparison of Malondialdehyde (MDA) according to pesticide exposure time of Spray Workers with control

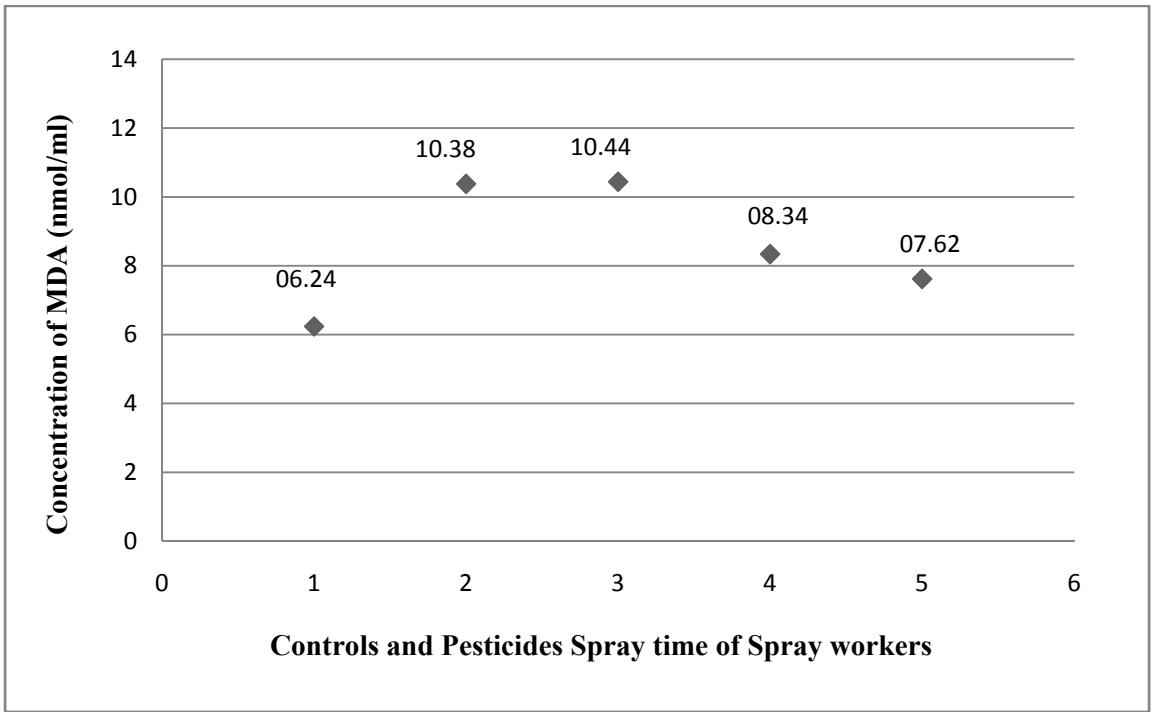

Fig. 2 Comparison of Malondialdehyde (MDA) according to pesticide Spray time of Spray Workers with control

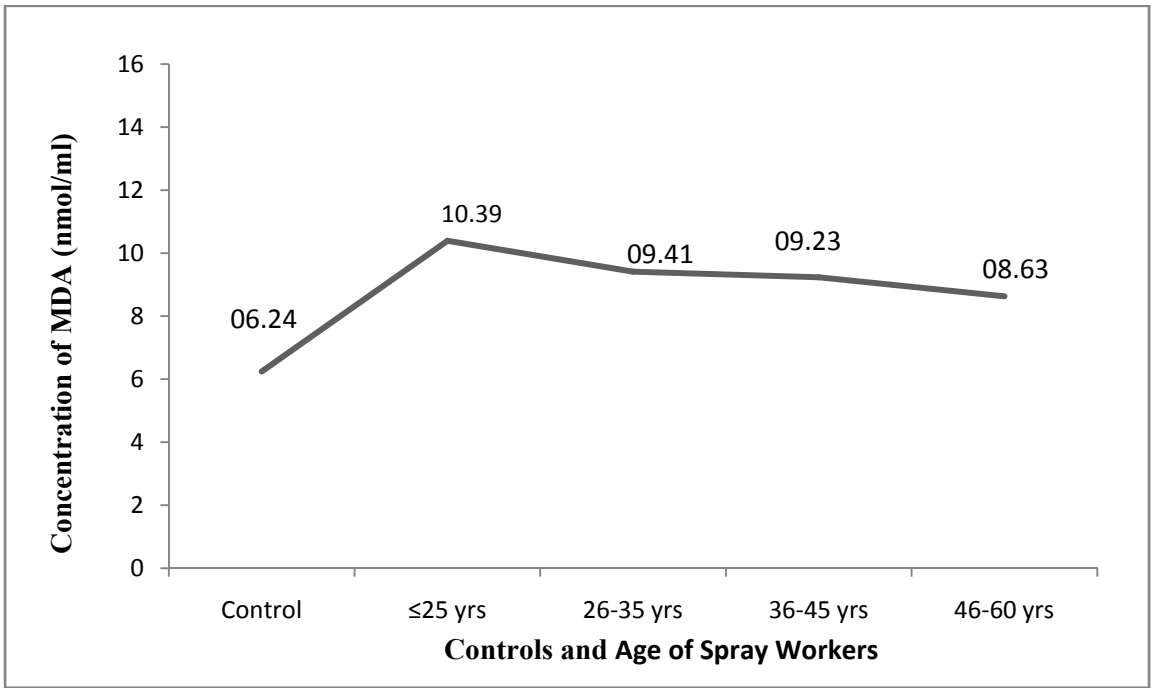

Fig. 3 Comparison of Malondialdehyde (MDA) according to age of Spray Workers with control

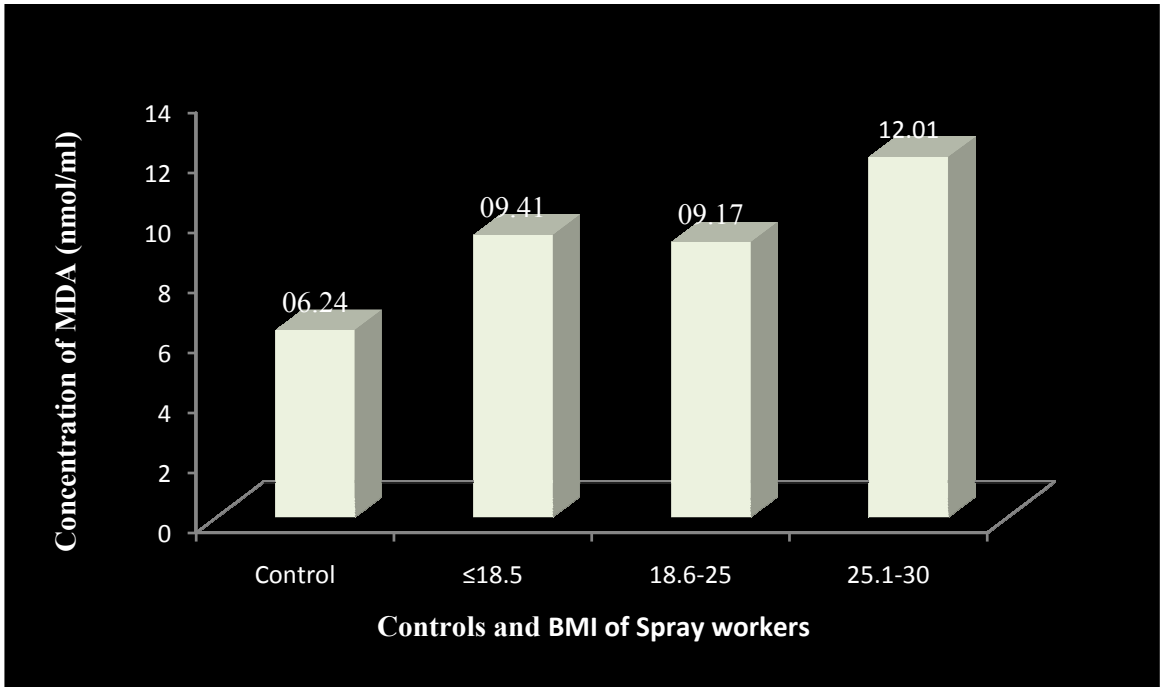

Fig. 4 Comparison of Malondialdehyde (MDA) according to Body Mass Index (BMI) of Spray Workers with control