

Possible Involvement of Paints Exposure and Smoking on Human Fertility

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Abstract:

In parallel with industrial advancements, number of the occupational diseases secondary to chemical exposure is increasing. Therefore, the aim of this study was to determine the effect of paints exposure and smoking on male infertility. 152 painters were collected and classified into non-smokers and smokers. The non-smokers were either exposed to paints for less than 15 years (GII) or for more than 15 years (GIII) and the smoker painters were either exposed for less (GIV) or more than 15 years (GV), in addition, control group (GI). In sera of the (GII - GV) the mean levels of testosterone were lowered than that of the (GI). On the other hand, the levels of estradiol (E2) in sera of all painters, groups were elevated than that of the control. Also, the mean levels of nitric oxide (NO), cadmium (Cd²⁺), lead (Pb²⁺), cobalt (Co²⁺), nickel (Ni²⁺) and copper (Cu²⁺) were elevated in GII - GV.

Key words: Occupational diseases, hormones, infertility, heavy metals and nitric oxide.

I-Introduction:

Technically speaking, infertility is the inability to become pregnant after having unprotected sexual intercourse regularly during ovulation for a period of twelve months or more, or the inability to carry a pregnancy to full term. Low sperm count, low motility, malformed sperm, or blocked sperm ducts are among the men problems. Also, men fertility can be adversely affected by exposure to radiation, pesticides, or other environmental poisons [38].

In parallel with industrial advancements, the number of the occupational diseases secondary to chemical exposure is increasing. This work environment usually contains a large number of chemicals, which may be inhaled and absorbed by the body, a process which can affect various organ and tissue systems leading to chronic diseases [47].

1.1. Paints and sex hormones:

In the last few years, there has been a growing interest and concern for the study of a heterogeneous group of environmental chemicals which may act as endocrine disruptors (EDCs) [23]. Many EDCs are classified as “xenoestrogen” because their action mimics that of estrogen hormones [49]. Multiple lines of evidence from several animal studies indicate that exposure to certain EDCs may drastically alter functional properties of the reproductive, immune, neurologic and endocrine systems [18, 21]. Exposure to environmental toxicants such as copper has been suggested to have adverse effects on male reproduction.

1.2. Heavy metals and infertility:

It has been hypothesized that exposure to environmental toxicants including heavy metals had been suggested to have adverse effects on male reproduction especially when humans are occupationally and environmentally exposed to their aerosols. Cd²⁺ is highly toxic metal for humans; it is pervasive in the human environment and accumulates in the human body over a lifetime. The biological half-life of Cd²⁺

in the human body is estimated to be about 10 - 20 years [40]. The concentration of Cd^{2+} in serum is regarded as the best biological indicator of recent exposure to Cd^{2+} . The majority of information concerning the adverse effects of Cd^{2+} on male reproductive capacity is related to experimental animal studies while data in human subjects are inconclusive [28]. Pb^{2+} is inevitably present in the human environment and is known to be toxic agents who accumulate in the human body over a lifetime including male reproductive organs [41]. Epidemiological studies have been equivocal about effects of Pb^{2+} and Cd^{2+} on hormone concentrations, male fertility and sperm parameters [8, 39].

In 1997, Paksy et al. showed that Zn + 2 it impaired fertility by 20%, while Cd+2 dose-dependently blocked the receptivity of female rats. In addition, when Zn⁺² and Cd⁺² were added to the culture medium of ovarian granulosa cells Cd⁺² suppressed follicle-stimulating-hormone-(FSH-) and cAMP-stimulated progesterone accumulation [34]. [36] Added that, the reduction in fertility at low exposure levels of Pb²⁺ could be explained by occupational exposure to solvents. In the time-to-pregnancy study among men exposed to organic solvents, however, occupational exposure to these agents was only suggestively associated with reduced fecund ability [37]. In addition, [36] reported that, the association between Pb²⁺ exposure and reduced fertility seemed to be stronger in older age groups. A similar finding was made in the Danish study [9] which showed that, exposure to Pb²⁺ was associated with reduced fertility among older men but not among younger men (cutoff point at 30 years of age) in a subset of battery workers with at least [Pb²⁺] >20 µg/dl. [44] Added that the mechanisms by which Zn⁺² and Cd⁺² can affect FSH-stimulated progesterone production seem to be different.

Cobalt is a suspected reproductive toxicant and exposure to it has the potential to negatively affect the human reproductive system. Cobalt is used in alloys, batteries, pigments, coloring agents, electroplating, and production of other chemicals and in various other applications. Studies in mice showed a decrease of sperm motility and/or an increase of abnormal sperm cells after inhalation, acute exposure, or dietary administration of [10].

1.3. Nitric oxide and infertility:

[4] showed that NO are implicated in the steroid hormones binding to albumin thus affecting free hormonal fractions a phenomenon which can explain somewhat the pathophysiological role NO and other free radicals in different steroid-hormones related diseases including human infertility.

NO is a free radical and a highly reactive central mediator in biological systems which is generated by NO synthase (NOS) [7]. Several studies have shown that this enzyme is associated with acrosome and tails of human sperm and it appears to be involved in sperm motility and acrosomal reaction that are the main factors in fertilization process [24, 14]. In addition, [19] observed a significantly decreased in sperm motility and a significantly increased MDA, nitrite, and nitrate concentrations in the sperm of normozoospermic smokers in comparison with non-smoker men.

Several reports support the negative effect of cigarette smoke on sperm motility in a concentration- and time-dependent manner [11, 26]. However, the literature concerning the effects of cigarette smoking and/or paints on sex hormones, thyroid stimulating hormone (TSH), heavy metals accumulation and NO concentration in fertile smoking men is limited. Therefore, the aim of this work was to illustrate if the exposure to paints and/or smoking can participate in human infertility or not.

2. Subjects and Methods

2.1. Subjects:

This study was conducted on 152 male painters with an age range of 16 - 58 years. The painters were collected from Elshoaraa, Elsinania and Elrawda regions, Damietta Governorate, Egypt. A group of 40 healthy males in the same age range were taken as controls. All individuals know the nature of the research and gave samples with consent.

The 152 painters were classified into non-smokers (GII and GIII) and smokers (GIV and GV). These painters were either exposed for paints for less than 15 years (GII and GIV) or for more than 15 years (GIII and GV), respectively.

2.1.2. Preparation of the blood samples:

3.0 ml of venous blood were collected from each painter and control subject and were left to clot at room temperature. After clotting, the samples were centrifuged and the serum samples were separated and stored at - 80 °C until their use.

2.2. Methods:

2.2.1. Enzyme-linked immuno-sorbent assay (ELISA):

A one-step enzyme linked immunosorbent assay (ELISA) was used for determination of testosterone according to the method of Marcus [31], Joshi *et al.*, [27] and Ekins [17]. Also, E2 was determined by ELISA according to the method of Tsang *et al.*, [42] and Gore-langton and Armstrong [20]. In addition, TSH was determined by ELISA according to the method of Hopton *et al.*, [25].

2.2.2. Colorimetric assays:

NO level was determined by the method of Montgomery *et al.*, [33].

2.2.3. Atomic absorption:

Cadmium, lead, cobalt, nickel and copper were determined in serum by atomic absorption spectrophotometer (Buck Scientific Accusys 211) according to the method of Rosner and Gorfien [35].

3-Results and discussions:

3.1. Serum testosterone levels: The mean level of testosterone of the healthy control was 26.0 ± 1.1 nmol/l (n=24) but that of the non-smoker painters with less than 15 years of exposure (GII) was 15.4 ± 1.94 nmol/l (n=36). In addition, the non-smoker painters of GIII showed a mean testosterone level of 12.7 ± 1.85 nmol/l (n=30). Generally, the mean levels of testosterone of the non-smoker painters (GII and GIII) were highly significantly decreased ($p < 0.001$) when compared with that of healthy control (**Table 1**).

The mean level of testosterone of the smoker painters with exposure time of less than 15 years (GIV) was 14.4 ± 2.4 nmol/l (n=27) but that of the painters who exposed for more than 15 years (GV) was 12.6 ± 2.17 nmol/l (n=27). Statistically, the mean testosterone levels of the smoker groups were highly significantly decreased ($p < 0.001$) than that of the control one (**Table 1**).

Normally, LH stimulates the testis to produce testosterone which stimulates testicular spermatogenesis [12]. Therefore, the reduction in serum testosterone levels of the painters who exposed for both paints and cigarettes smoke may cause defects in the process of spermatogenesis and thence infertility. In addition, [15] showed that in correlation with LH and FSH gonadotropins, testosterone in men regulates the sexual functioning and controls sexual desire, sexual development,

and semen production and maturation in the testes. Therefore, the long term exposure to cigarrate smoke and paints, in this study, can cause chronic alterations of E₂ and testosterone hormones and thence can harm both physical and sexual aspects of male anatomy including erectile dysfunction, low sperm production, and finally poor male fertility.

3.2. Estradiol level:

The mean level of E₂ of the healthy control (group I) was 51.0±6.8 pg/ml (n=21). Also, the level of E₂ of the non-smoker painters who were exposed for less than 15 years (GII) was 100.5±11.9 pg/ml (n=18) and that of whom who were exposed for more than 15 years (GIII) was 121.5±13.6 pg/ml (n=26). The statistical calculations of the latter mean values showed that the level of E₂ hormone was greatly and significantly elevated than that of the control group (**Table 1**).

With respect to the E₂ levels of the smoker painters (GIV and GV), it showed higher elevation in GV than that of GIV (mean=111.1 ± 13.9 pg/ml and 126.3 ± 12.3 pg/ml, respectively when compared with that of the healthy control (p<0.001) (**Table 1**).

In addition, E₂ has been shown to directly affect steroidogenesis in the rat testis via accumulation of estrogen-regulated proteins [43]. Therefore, one can expect that the elevation of E₂ levels in sera of the painters included in this study may stimulates Leydig cell hyperplasia which has been associated with cryptorchidism, testicular damage, and impaired spermatogenesis as was previously reported by [1] who found association between E₂ level and Leydig cell hyperplasia in rodents. Since [44, 16] demonstrated that, circulating LH and FSH concentrations were effectively reduced by E₂, therefore, the feedback mechanism of the elevated level of E₂ in this study can amplify the reduction of testosterone level possibly via LH and FSH mediated mechanism. This finding confirm that of [46] who found that paint thinner exposure for 15 days had decreased serum LH and testosterone concentrations in a parallel manner.

In addition to the effect of paints, the results of the present study confirm the negative effect of cigarette smoke on human fertility at least in part via reduction of serum testosterone level or via its negative effect on sperm motility in a concentration- and time-dependent manner. Somewhat similar results were previously reported by [11, 26].

3.3. Thyroid stimulating hormone (TSH):

The statistical analysis of the results showed that the mean level of thyroid-stimulating hormone (TSH) of the GII, GIII, GIV and GV were highly significantly increased (p<0.001) when compared with that of healthy control group (**Table 2**).

3.4. Heavy metals:

The mean serum levels of Cd²⁺ were elevated by 55.6%, 86.1%, 94.4%, and 119.4%, lead by 90.4%, 379.5%, 495.9% and 653.4%, cobalt by 140.5%, 153.2%, 279.7% and 406.3%, nickel by 23.3%, 33.3%, 113.3% and 123.3% and copper by 14.6%, 22.0%, 26.8% and 63.4% in GII, GIII, GIV and GV, respectively (**Table 3**).

In our results, the mean levels of Cd²⁺ in non-smoker painters (GII and GIII) were significant increased compared to GI. These results showed that paints can cause elevation of serum Cd²⁺ ions. In addition to the effect of paints, the results of the present study confirm the effect of cigarette smoke on serum Cd²⁺ levels this is because smoker painters (GIV and GV) showed very significant increases in their mean serum contents of Cd²⁺ than those of the non-smokers. Therefore these results confirm those of [13] at least in part on the effect of cigarette smoke-years

on blood Cd^{2+} on both sperm density and sperm volume, especially in heavy smokers. Based on these results, one can add that Cd^{2+} , which is present in cigarettes, could be a possible causative agent for lowering sperm density among smoker's painters. Unfortunately, the seminal parameters were not evaluated in the present study but the reduction in serum testosterone and the elevation of E2 mean levels after exposure to cigarette smoke and/or paints are direct evidence of testicular damage. This is because [32], found low sperm count, sperm motility, sperm maturity, and lowered level of testosterone during administration of a high dose of Cd^{2+} to the mice and attributed their finding to the severe necrosis and atrophy in the testis of the high dose exposed group, a process which showed no successful mating in such group. These authors concluded that, Cd^{2+} affects male reproductive system activity and can cause infertility in mice as an animal model. Moreover, calcium (Ca^{2+}) channels are susceptible to Cd^{2+} poisoning and potassium (K^+) channels to Pb^{2+} . These channel isoforms have been identified in human testes and spermatozoa and not only offer entry paths for metallic toxicants into mature spermatozoa but also are involved in the early events of acrosome reactions [8]. Therefore, this may be one of the mechanisms by which the excessive levels of both Cd^{2+} and Pb^{2+} in this study can contribute to human infertility. In addition, the authors render the deleterious effect of Cd^{2+} and other heavy metal ions on spermatogenesis to be mediated through their effects on reducing ascorbic acid level which finally induced oxidative damage on rat testis [29]. Also, [44] added that Zn^{2+} itself impaired fertility by 20%, while Cd^{2+} dose-dependently blocked the receptivity of female rats. They also added that, when Zn^{2+} and Cd^{2+} were added to the culture medium of ovarian granulosa cells, Cd^{2+} suppressed FSH- and cAMP-stimulated progesterone accumulation. This may be one of the infertility mechanisms which can be involved in this study at least in part by reducing testosterone level via the reduced FSH level. Also, no protective effect of Zn against Cd^{2+} -induced drop in progesterone production but Zn^{2+} by itself induced a significant increase in FSH-supported progesterone synthesis. On the other hand, Zn^{2+} by itself are not able to induce significant increase in FSH-supported progesterone synthesis. The authors concluded that, while Zn protected against Cd-induced sterility in vivo, it failed to counteract the direct effect of Cd on steroid biosynthesis.

[45] Showed that the relationship between the increase in urinary Ni and the increase in plasma FSH may depends on the action of the former, even at low doses, on the hypothalamic-pituitary-gonadal axis. The increases in serum Ni in this study confirm the previous findings.

[22], found that, hyperthyroidism or hypothyroidism are associated with altered fertility due to changes in the levels and activities of hormones of the brain-pituitary-gonadal axis. [22], added that, multi factorial control of reproduction invariably involves regulation of metabolism by a number of hormones including TSH, which are known to have profound effect on body metabolism, growth and development. Also, there are various reports indicating that thyroid hormones interact with the hormones of brain-pituitary-gonadal axis. [30], added that normal thyroid function is important for normal regulation of reproduction. Based on the results of the present study, it was suggested that the increase in the mean heavy metals levels not only directly participate in human infertility via inhibiting sex hormones but also through their indirect effect which include thyroid hormones or their trophic hormone; namely, TSH. The increase in the mean level of TSH with simultaneous reductions in the mean levels of testosterone and the results of [5] which showed that urinary cadmium was associated with increases in both T3 and T4 [2] confirms the previous suggestion.

The Cu/Zn ratio in seminal plasma was significantly ($p < 0.01$) higher in controls compared with oligozoospermic and azoospermic patients [3]. [6] Added that, copper administration to rats caused a significant damage to seminiferous tubules diameter, spermatogonial cells nuclei diameter, sertoli cells nuclei diameter and epithelial height compared to the non-exposed rats.

3.5. Nitric oxide:

The mean level of nitric oxide of the healthy control (group I) was 4.2 ± 0.25 $\mu\text{mol/l}$ ($n = 28$). In the non-smoker painters (less than 15 years, group II), the mean level of nitric oxide was 18.0 ± 1.6 $\mu\text{mol/l}$ ($n = 35$). In addition, the non-smoker painters (more than 15 years, group III) showed a mean value of nitric oxide of 24.5 ± 2.2 $\mu\text{mol/l}$ ($n = 26$).

The mean values of nitric oxide of the GII and GIII were highly significantly increased ($p < 0.001$) when compared with that of the healthy control group (**Table 2**).

The mean level of nitric oxide of the smoker painters (less than 15 years, GIV) was 23.0 ± 1.5 $\mu\text{mol/l}$ ($n = 21$). Also, a higher level of nitric oxide was found in sera of the smoker painters (more than 15 years, GV) 25.7 ± 1.9 $\mu\text{mol/l}$ ($n = 42$).

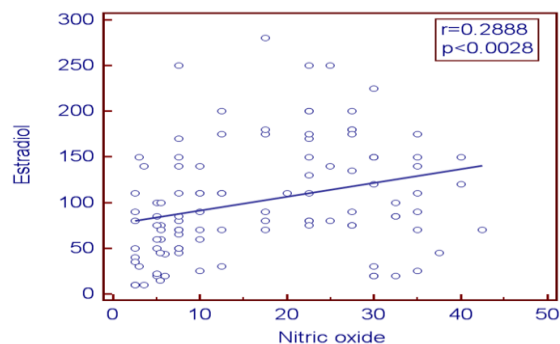
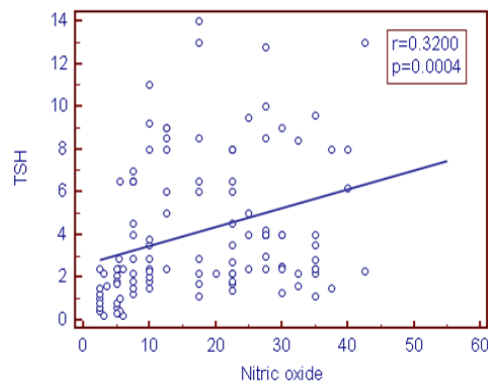
The mean values of nitric oxide of the GIV and GV were highly significantly increased ($p < 0.001$) when compared with that of healthy control (**Table 2**).

[48], showed NO plays a crucial role in reproduction at every level in the organism. In the brain, it activates the release of luteinizing hormone-releasing hormone (LHRH). The axons of the LHRH neurons project to the mating centers in the brain stem.

4-Correlation:

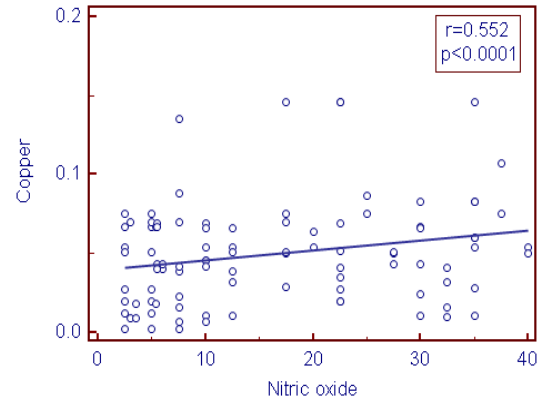
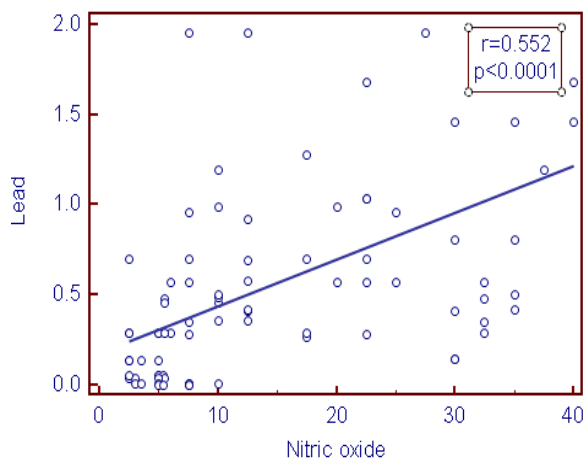
4.1. Nitric oxide and hormones:

Nitric oxide has a positive correlation with estradiol ($r = 0.2888$, $p < 0.0028$) and TSH ($r = 0.3200$, $p = 0.0004$).



4.2. Nitric oxide and heavy metals:

Nitric oxide has a positive correlation with copper ($r=0.2223$, $p=0.0304$) and lead ($r=0.552$, $p<0.0001$).



4.3. Heavy metals and hormones:

Cadmium was negatively correlated with testosterone ($r=-0.2468$, $p=0.0263$). In addition, lead was positively correlated with TSH ($r=0.3709$, $p=0.0006$).

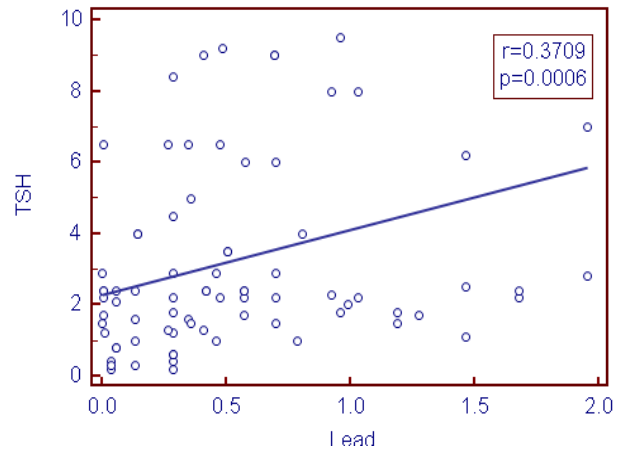
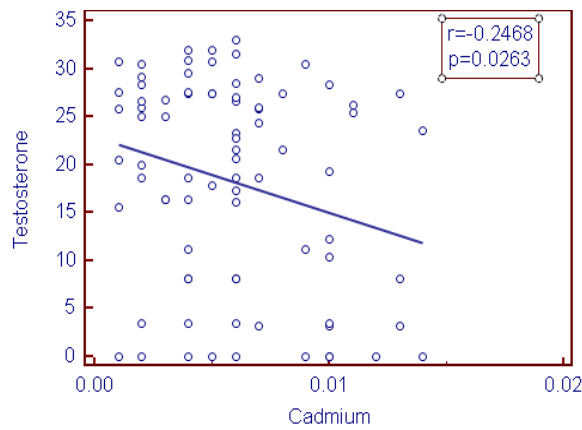


Table 1: The mean serum levels of testosterone and E₂ of non-smoker and smoker painters who were exposed for paints either for less than 15 years or for more than 15 years compared to those of the healthy control.

Parameter	Non-Smokers painters		Smokers painters	
	Testosterone (nmol/l)	Estradiol (E ₂) (pg/ml)	Testosterone (nmol/l)	Estradiol (E ₂) (pg/ml)
Control				
Mean ± S.E	26.0 ± 1.1	51.0 ± 6.8	26.0 ± 1.1	51.0 ± 6.8
n	24	21	24	21
Less than 15 year				
Mean ± S.E	15.4 ± 1.94***	100.5 ± 11.9***	14.4 ± 2.4***	111.1 ± 13.9***
n	36	18	27	18
Percent	[40.8%] ^a	[97.1%] ^b	[44.6%] ^a	[117.8%] ^b
More than 15 year				
Mean ± S.E	12.7 ± 1.85***	121.5 ± 13.6***	12.6 ± 2.17***	126.3 ± 12.3***
n	30	26	27	26
Percent	[51.2%] ^a	[138.2%] ^b	[51.5%] ^a	[147.6%] ^b

- Values were expressed as mean ±Standard error (S.E) in each case.

*= Significant, **=very significant and ***= extremely significant when compared with the corresponding values of control.

[]^a = Percent of reduction and []^b = Percent of elevation than those of the control.

[]^a & []^b=(Mean of the control – Mean of the tested group)/Mean of the control.

Table 2: The mean serum levels of TSH and nitric oxide of non-smoker and smoker painters who were exposed for paints either for less than 15 years or for more than 15 years compared to those of the healthy control.

Parameter	Non-Smokers painters		Smokers painters	
	TSH	Nitric oxide	TSH	Nitric oxide
Control				
<i>Mean ± S.E</i>	1.0 ± 0.18	4.2 ± 0.25	1.0 ± 0.18	4.2 ± 0.25
<i>n</i>	17	28	17	28
Less than 15 year				
<i>Mean ± S.E</i>	2.79 ± 0.36^{***}	18.0 ± 1.6^{***}	5.6 ± 0.57^{***}	23.0 ± 1.5^{***}
<i>n</i>	24	35	21	21
<i>Percent</i>	[179.0%]^a	[328.6%]^a	[460.0%]^a	[447.6%]^a
More than 15 year				
<i>Mean ± S.E</i>	4.4 ± 0.53^{***}	24.5 ± 2.2^{***}	5.98 ± 0.77^{***}	25.7 ± 1.9^{***}
<i>n</i>	33	26	27	42
<i>Percent</i>	[340.0%]^a	[483.3%]^a	[498.0%]^a	[511.9%]^a

- Values were expressed as mean ±Standard error (S.E) in each case.

*= Significant, **=very significant and ***= extremely significant when compared with the corresponding values of control.

[]^a = Percent of reduction and []^b = Percent of elevation than those of the control.

[]^a & []^b=(Mean of the control – Mean of the tested group)/Mean of the control.

Table 3: The mean serum levels of cadmium, lead, cobalt, nickel and copper of non-smoker and smoker painters who were exposed for paints either for less than 15 years or for more than 15 years compared to those of the healthy control.

Group Parameter	Less than 15 year		More than 15 year		control
	Non-Smokers	Smokers	Non-Smokers	Smokers	
Cadmium <i>Mean ± S.E</i> <i>n</i> <i>Percent</i>	0.56 ± 0.06* 20 [55.6%] ^b	0.7 ± 0.09** 14 [94.4%] ^b	0.67 ± 0.096* 18 [86.1%] ^b	0.79 ± 0.07** 24 [119.4%] ^b	0.36 ± 0.06 12
Lead <i>Mean ± S.E</i> <i>n</i> <i>Percent</i>	27.8 ± 5.8* 22 [90.4%] ^b	87.0 ± 14.9*** 17 [495.9%] ^b	70.0 ± 8.0*** 18 [379.5%] ^b	110 ± 15.9*** 14 [653.4%] ^b	14.6 ± 2.5 18
Cobalt <i>Mean ± S.E</i> <i>n</i> <i>Percent</i>	1.9 ± 0.1*** 16 [140.5%] ^b	3.0 ± 0.87* 13 [279.7%] ^b	2.0 ± 0.36** 16 [153.2%] ^b	4.0 ± 0.4*** 12 [406.3%] ^b	0.79 ± 0.14 20
Nickel <i>Mean ± S.E</i> <i>n</i> <i>Percent</i>	3.7 ± 0.75 14 [23.3%] ^b	6.4 ± 1.5 10 [113.3%] ^b	4.0 ± 0.87 15 [33.3%] ^b	6.7 ± 0.84** 12 [123.3%] ^b	3.0 ± 0.66 10
Copper <i>Mean ± S.E</i> <i>n</i> <i>Percent</i>	4.7 ± 0.87 18 [14.6%] ^b	5.2 ± 0.5 19 [26.8%] ^b	5.0 ± 0.59 15 [22.0%] ^b	6.7 ± 1.2 16 [63.4%] ^b	4.1 ± 0.45 30

- Values were expressed as mean ± Standard error (S.E) in µg/dl each case.

*= Significant, **=very significant and ***= extremely significant when compared with the corresponding values of control.

[] a = Percent of reduction and [] b = Percent of elevation than those of the control.

[] a & [] b = (Mean of the control – Mean of the tested group)/Mean of the control.

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