Evaluation of Some Parameters For Workers in Gas Filling Company - Branch Middle Euphrates / Hilla Gas Factory Via Oxidant-Antioxidant System

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Abstract:

This study included determination of Malondialehyde, Peroxynitrite, vitamin C and vitamin E ,for workers in gas filling company - Branch Middle Euphrates / Hilla Gas factory to investigate the effect of LPG and additive that use to give special properties (smell) thiophene .The workers divided into two groups, the first group (workers of empatting), the second group (workers of equipment) . The workers divided into three groups based upon the period working at a factory (three months–one year , 2–6 , 7–13) years.The results of this research explain increasing levels of MDA and peroxynitrite while decreasing of vit C and vit E . All this results are indictor for influence of LPG on the workers healthy.

Key words : Malondialehyde ; Peroxynitrite ; vitamin C ; vitamin E .

الخلاصة:

الدراسة تتضمن تقدير كل من مالون ثنائي الديهايد وبيروكسي نتريت وفيتامين C وفيتامين E لعمال في معمل تعبئة الغاز فرات الاوسط / معمل غاز الحلة لتحقيق تأثير الغاز البروبان السائل والمادة المضافة لعطائه صفات خصوصية (رائحة) ثايوفين . العمال قسموا الى مجموعتين مجموعة الاولى (عمال التعبئة), مجموعة الثانية (عمال المعدات) . العمال قسموا الى ثلاث مجاميع على أساس فترة العمل في المعمل (ثلاث أشهر – سنة , 2–6 , 7– 13) سنة . النتيجة من هذه الدراسة زيادة في مستويات مالون ثنائي الديهايد وبيروكسي نتريت وبينما نقصان في فيتامين C وفيتامين E كل هذه النتائج هي دليل لتأثير الغاز البروبان السائل على صحة العمال .

الكلمات المفتاحية: مالون ثنائي الديهايد ، بيروكسي نتريت ، فيتامين C ، فيتامين E

Introduction:

Liquefied petroleum gas (LPG) is a group of hydrocarbons derived from raw petroleum processes liquefied petroleum gas (LPG) is known as a hydrocarbon borderline product (Leary,1980).The components of the liquefied petroleum gas (LPG) (97.8%propane, 1.5% isobutane, 0.1% n-butane, 0.2% propylene, and 0.4% other gases) (Ago et al.,2002; Fuke et al.,2002) .The relationship between the environment and the concept of oxidative stress and based on the scientific prove that free radicals and oxidative stress are critical importance in the role of these agents play in various biochemical aspects during the life, like diseases, adaptive. changes and physiological homeostatic (Ames and Shigenaga,1992).

Free Radicals are molecules with an unpaired electron they contain an odd number of electrons, due to the presence from a free electron, these molecules are highly reactive , very unstable react quickly with other compounds and try to capture the needed electron to gain stability (Moad and Solomon ,1995)(Jaideep *et al* ., 2012).Reactive oxygen species are induced by substances such as transitional metal ions, pesticides, and petroleum pollutants (Slaninova *et al.,* 2009; Lushchak, 2011).The lipid peroxidation is considered as the most damaging process known that membrane damage of every living organism, Lipid peroxidation are formed from polyunsaturated precursors result of oxidative stresses (Garg and Manchanda, 2009).Suggested Mechanism that MDA is formed through react radical thiophene with unsaturated fatty acid of several steps Modification From (Halliwell and Gutteridge,1999).

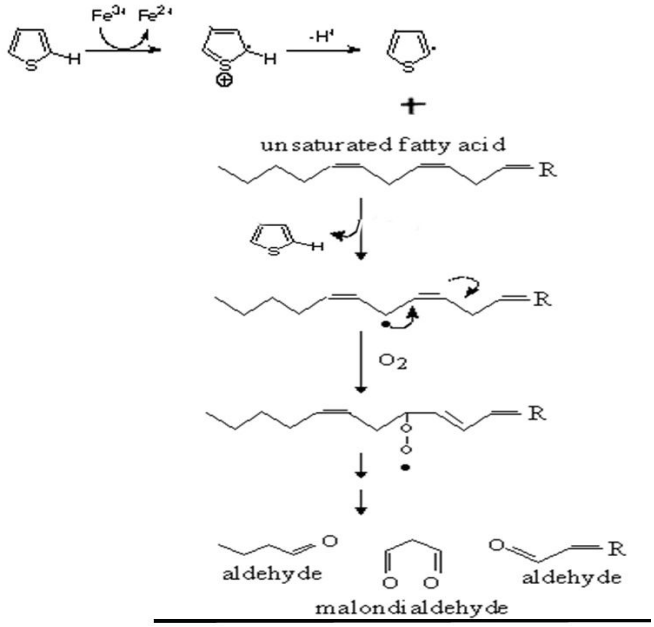

Fig.(1-1) Suggested Mechanism of Reacting Thiophene with Unsaturated Fatty Acid Modification From (Halliwell and Gutteridge, 1999).

Peroxynitrite is formed in biological systems of superoxide and nitric oxide are produced at near equimolar ratio, is reactive nitrogen species and an anion with the formula ($ONOO^-$) peroxynitrite is powerful oxidant exhibiting a wide array from tissue damaging effects ranging from lipid peroxidation, inactivation of enzymes and ion channels via protein oxidation and nitration to inhibition of mitochondrial respiration(Virag *et al* ., 2003).

An antioxidant is a substance able to delay or slow the oxidation of other molecules by restraining the initiation or propagation from an oxidation chain reaction (Ramle SFM et al, 2008; Boxin *et al* ., 2002). Ascorbic acid or "vitamin C" is a monosaccharide antioxidant found in both animals and plants, in cells, it maintained in its reduced form by reaction with glutathione, which can be catalyzed by protein disulfide isomerase, Ascorbic acid is a reducing agent and can reduce and thereby neutralize, reactive oxygen species such as hydrogen peroxide (Bjelakovic *et al.,* 2007; Ortega, 2006).Vitamin E (tocopherols) its lipid soluble antioxidant and considered as potential scavengers of ROS and lipid radicals, and it considered as a major antioxidant in biomembranes, its play both antioxidant and non-antioxidant functions, tocopherols are considered general antioxidants for protection of membrane stability, including quenching or scavenging ROS (Sarvajeet and Narendra, 2010).

Subjects:

The workers of Middle Euphrates / Hilla Gas factory divided into two groups.Workers of plant exposure to variouse of the environmental impact exposure them to gases (specially gases empatting unity) ,exposure them to thiophene (within empatting unity), and another workers of plant exposure to gases and to acids wich using of valves washing (equipment unity).Therefor workers divided in plant to two groups , the first group is the workers who work in (empatting unity), and another is the workers who work in (equipment unity).The workers divided into three groups based upon the period working at factory (the first group (G1):three months–one year , the second group(G2): 2–6 , the third group(G3): (7–13) years. This study included collection of blood samples from Middle Euphrates Gas plant workers (males) in Hilla city ,Who worked in the plant for period of time ranging from (1-15 years) and their ages between (17-40 years) compared to a control group wasn't indirect exposure to

any kind of chemicals. The study has also included a questionnaire about the worker criteria : Age, Period of working, Smokers , and any chronic diseases, skin diseases and headache.

Methods :

Determination of Malondialdehyde:

The principle of the following method was based on the spectrophotometric measurement of the color, occurred during the reaction between thiobarbituric acid (TBA) and MDA, (Burtis and Ashwood, 1999). read the absorbance of sample at 532nm .

Determination of Peroxinitrate :

Sample which contain peroxynitrate and added to phenol in 50 mM sodium phosphate buffer pH 7.4 mediated nitration of phenol, after incubation for 2h at 37 ^{o}c, NaOH was add to produce the salt of nitrophenol, which has maximum absorbance at 412 nm. The yield of nitrophenol was calculated from 4400 M^{-1} cm^{-1}.(Beckman *et al.,* 1992).

Determination of ascorbic acid (vitamin C):

The chemical methods which are available for assessment of ascorbic acid are depend on either the reducing properties of the 1,2-enediol group that lead to absorbance changes in indicator dyes or formation of hydrozone, the 2,4-dinitrophenyl hydrazine (DNPH) methods, ascorbic acid (AA) is oxidized by Cu^{+2} to dehydroascorbic acid (DHA) and diketogulonic acid (Tietz, 1995). When treated with DNPH, the 2,4-dehydrophenyl osazon product forms which in the presence of sulfuric acid (Burtis and Ashwood, 1999), forms an orange red complex that absorbed at 520 nm .

Determination of vitamin E:

Proteins in the plasma or serum are precipitate by an equal volume of absolute ethanol, the whole mixture is subjected to extraction by an equal volume of xylene. The α, α-dipyridyl is added to an aliquot of the upper layer to estimate the principle interfering substance, at 460nm. At this time the ferric chloride ($FeCl_3$) reagents are added to system to produce the color obtained by the Emmerie-Engel procedure which is measured at 520nm (Hashim and Schuttringer, 1966).

Statistical Analysis:

Statistical analysis was performed by Microsoft excel office 2010 and SPSS statistics. Subjects with groups were compared among them . Means, standard deviation , SE , confidence interval 95% (lower and upper) the level of significance was set at $P \leq 0.05$.

Discussion and Results :

This study included the investigation of LPG influence in Iraq workers healthy by determination of oxidants such as Malondialehyde, Peroxynitrite as well as assesement of antioxidants such as vitamin C, vitamin E. The result of this study explain increasing of MDA levels for excessive formation of free radicals results in an increase in the process of lipid peroxidation,as evidenced by elevated levels of malondialdehyde (MDA), the end product of lipid peroxidation, of plasma and tissues (Baglia *et al.*,1997; Nada, 2007). Therefor MDA is one of the major oxidation products from peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of LPO (Hasan *et al* ., 2013). From the table (1) shows a significant increase in MDA levels for workers of place groups and workers of period groups compared with control.From the table (2) shows a significant increase in MDA levels for workers empatting of period and Workers equipment of period compared with control..as show in Table (1) and (2).

Table (1) MDA (µmole/L) level for gas workers compared with control.

The groups		N	Mean ± SD µmole/ L	SE	95% CI		P-value
					Lower	Upper	
workers of place groups	GT	60	4.54 ± 1.08	0.14	1.84	7.19	0.000*
	G1	30	4.98 ± 1.06	0.19	2.19	7.19	0.000*
	G2	30	4.11 ± 0.92	0.17	1.84	5.67	0.000*
							0.007**
workers of period groups	G1	17	4.64 ± 0.93	0.22	3.48	7.17	0.000*
							0.795****
	G2	23	4.82 ± 1.31	0.27	2.10	7.65	0.000*
							0.958**
	G3	20	4.88 ± 0.93	0.21	2.19	7.17	0.000*
							0.881**
							0.819***
	Control	42	1.43 ± 0.33	0.05	1.03	2.85	--------

A : workers of place groups:-

*: its mean significance related of total group or group 1 or group 2 with control group.**: its mean significance related of group 1 with group 2.

B : workers of period groups:-

*: its mean significance related of group 1 or group 2 or group 3 with control group . **: its mean significance related of group 1with group 2 or group 1 with group 3 . ***: its mean significance related of group 2 with group 3. ****: its mean significance related of group1 with group 2 with group 3.

Table (2) MDA (µmole/L) level for gas workers (empatting and equipment) of the period compared with control.

	The groups	N	Mean ± SD µmole/ L	SE	95% CI		P-value
					Lower	Upper	
Workers empatting of period	G1	12	4.48 ± 0.67	0.19	4.05	4.91	0.000*
							0.673****
	G2	11	4.55 ± 1.34	0.40	3.79	5.31	0.000*
							0.727**
	G3	9	4.63 ± 0.98	0.32	3.73	5.53	0.000*
							0.397**
							0.078***
Workers equipment of period	G1	5	4.98 ± 1.38	0.61	3.33	6.76	0.000*
							0.941****
	G2	12	5.05 ± 1.31	0.35	2.10	7.65	0.000*
							0.993**
	G3	11	5.45 ± 0.84	0.25	2.19	7.17	0.000*
							0.599**
							0.102***
	Control	42	1.43 ± 0.33	0.05	1.03	2.85	--------

C: workers of period groups (empatting and equipment) :-

*: its mean significance related of group 1 or group 2 or group 3 with control group . **: its mean significance related of group 1with group 2 or group 1with group 3 . ***: its mean significance related of group 2 with group 3. ****: its mean significance related of group1 with group 2 with group3.

While results suggested elevating in the peroxynitrite level caused by exposure to LPG . Elevating $NO^{\cdot}$ with superoxide anion indicates the production of peroxynitrite, a highly toxic anion from peroxidation, peroxynitrite is not a free radical because the un-paired electrons of $NO^{\cdot}$ and $O2^{\cdot-}$ combine to create a new N-O bond in peroxynitrite. However, peroxynitrite has a strong one- or two-electron oxidant and acts as nitrating agent. (Loganathasamy, 2012).From the table (3) shows a significant increase in peroxynitrite levels for workers of place groups and workers of period groups compared with control. From the table (4) shows a significant increase in peroxynitrite levels for workers empatting of period and Workers equipment of period compared with control. as show in Table (3) and (4).

Table (3) Peroxynitrite (µmole/ L) levels for gas workers compared with control.

The groups		N	Mean ± SD µmole/ L	SE	95% CI		P-value
					Lower	Upper	
workers of place groups	GT	60	82.00± 22.79	2.94	47.27	190.00	0.000*
	G1	30	80.56 ± 30.34	5.54	47.27	190.00	0.000*
	G2	30	83.00 ± 11.55	2.11	56.00	113.00	0.000*
							0.692**
workers of period groups	G1	17	74.09 ± 15.23	3.69	47.27	94.00	0.000*
							0.044****
	G2	23	83.21 ± 9.10	1.89	66.00	96.00	0.000*
							0.031**
	G3	20	83.96 ± 34.43	7.71	56.00	190.00	0.000*
							0.139 **
							0.781***
	Control	42	4.38 ± 1.76	0.27	2.42	8.63	---------

A : workers of place groups:-

*: its mean significance related of total group or group 1 or group 2 with control group. **: its mean significance related of group 1 with group 2.

B : workers of period groups:-

*: its mean significance related of group 1 or group 2 or group 3 with control group . **: its mean significance related of group 1with group 2 or group 1 with group 3 . ***: its mean significance related of group 2 with group 3. ****: its mean significance related of group1 with group 2 with group 3.

Table (4) Peroxynitrite (µmole/ L) level for gas workers (empatting and equipment) of the period compared with control.

The groups		N	Mean ± SD µmole/ L	SE	95% CI		P-value
					Lower	Upper	
kers empatting of period	G1	12	73.83 ± 7.65	2.21	68.97	78.69	0.000*
							0.292****
	G2	11	83.18 ± 7.26	2.19	78.14	86.77	0.000*
							0.020**
	G3	9	100.00 ± 46.41	15.47	63.99	135.35	0.000*
							0.137**
							0.269***
Workers equipment of period	G1	5	60.11 ± 14.05	6.28	42.65	77.55	0.001*
							0.088****
	G2	12	73.75 ± 6.19	1.78	63.49	76.87	0.000*
							0.133**
	G3	11	81.27 ± 11.98	3.61	78.26	92.41	0.000*
							0.0524**
							0.104***
	Control	42	4.38 ± 1.76	0.27	2.42	8.63	--------

C: workers of period groups (empatting and equipment) :-

*: its mean significance related of group 1 or group 2 or group 3 with control group . **: its mean significance related of group 1with group 2 or group 1with group 3 . ***: its mean significance related of group 2 with group 3. ****: its mean significance related of group1 with group 2 with group3.

The results shown decrease in vitamin E level for workers caused by exposure to LPG . Shown studies that oxidative stress causes a rapid depletion of vitamin E and consider α-Tocopherol as a defense substrate against peroxynitrite and lead to increasing from lipid peroxidation (Hoeldtk *et al* ., 2002; Bouayed and Bohn, 2010). From the table (5) shows a significant decrease in vitamin E levels for workers of place groups and workers of period groups compared with control. From the table (6) shows a significant decrease in vitamin E levels for workers empatting of period and equipment of period compared with control. Shown that vitamin E is decrease with the increase of the worker's period factor. This study indicated that serum vitamin E levels was significant reduced in workers compared with control $P \leq 0.05$.as show in Table (5) and (6).

Table (5) Vitamin E (mg/dL) level for gas workers compared with control.

	The groups	N	Mean ± SD (mg/dL)	SE	95% CI		P-value
					Lower	Upper	
workers of place groups	GT	60	0.585 ± 0.141	0.018	0.330	0.980	0.000*
	G1	30	0.581 ± 0.168	0.031	0.333	0.980	0.000*
	G2	30	0.584 ± 0.111	0.021	0.330	0.900	0.000*
							0.922**
workers of period groups	G1	17	0.601 ± 0.134	0.032	0.452	0.890	0.000*
							0.627****
	G2	23	0.541 ± 0.118	0.024	0.392	0.820	0.000*
							0.244**
	G3	20	0.517 ± 0.073	0.016	0.330	0.732	0.000*
							0.139**
							0.146***
	Control	42	9.262 ± 0.765	0.118	7.050	11.260	---------

A : workers of place groups:-

*: its mean significance related of total group or group 1 or group 2 with control group. **: its mean significance related of group 1 with group 2.

B : workers of period groups:-

*: its mean significance related of group 1 or group 2 or group 3 with control group . **: its mean significance related of group 1with group 2 or group 1with group 3 . ***: its mean significance related of group 2 with group 3. ****: its mean significance related of group1 with group 2 with group 3.

Table (6) Vitamin E (mg/dL) level for gas workers (empatting and equipment) of period compared with control.

	The groups	N	Mean ± SD (mg/dL)	SE	95% CI		P-value
					Lower	Upper	
kers empatting of period	G1	12	0.521 ± 0.092	0.026	0.484	0.601	0.000*
							0.313****
	G2	11	0.504 ± 0.073	0.022	0.455	0.553	0.000*
							0.029**
	G3	9	0.485 ± 0.048	0.016	0.448	0.523	0.000*
							0.034**
							0.299***
Workers equipment of period	G1	5	0.754 ± 0.108	0.048	0.619	0.889	0.001*
							0.227****
	G2	12	0.610 ± 0.065	0.019	0.568	0.652	0.000*
							0.005**
	G3	11	0.572 ± 0.077	0.023	0.520	0.624	0.000*
							0.025**
							0.346***
	Control	42	9.262 ± 0.765	0.118	7.050	11.260	--------

C: workers of period groups (empatting and equipment) :-

*: its mean significance related of group 1 or group 2 or group 3 with control group . **: its mean significance related of group 1with group 2 or group 1with group 3 . ***: its mean significance related of group 2 with group 3. ****: its mean significance related of group1 with group 2 with group3.

Vitamin C functions as an antioxidant by reacting directly with ROS or regenerating vitamin E of α- tocopheroxyl radical, thus depletion of serum vitamin C workers of gas is due to the increased oxidative stress (Bursell *et al.,* 1999). Shown studies have that oxidative stress causes a rapid depletion from vitamin C(Bouayed and Bohn, 2010). That show vitamin C is decrease with the increase of the worker's period factor. There was a significantly decreased vitamin C levels in serum workers than that of control .From the table (7) shows a significant decrease in vitamin C levels for workers of place groups and workers of period groups compared with control. From the table (8) shows a significant decrease in vitamin C levels for workers empatting of period and workers equipment of period compared with control. as show in Table (7) and (8).

Table (7) Vitamin C (mg/dL) level for gas workers compared with control.

	The groups	N	Mean ± SD mg/dL	SE	95% CI		P-value
					Lower	Upper	
workers of place groups	GT	60	0.133 ± 0.025	0.0032	0.087	0.275	0.000*
	G1	30	0.134 ± 0.021	0.0034	0.100	0.200	0.000*
	G2	30	0.132 ± 0.029	0.0052	0.087	0.275	0.000*
							0.829**
workers of period groups	G1	17	0.145 ± 0.027	0.006	0.119	0.275	0.000*
							0.155****
	G2	23	0.131 ± 0.034	0.007	0.093	0.200	0.000*
							0.961**
	G3	20	0.120 ± 0.023	0.005	0.087	0.136	0.000*
							0.211**
							0.671***
	Control	42	0.804 ± 0.370	0.057	0.540	1.920	---------

A : workers of place groups:-

*: its mean significance related of total group or group 1 or group 2 with control group. **: its mean significance related of group 1 with group 2.

B : workers of period groups:-

*: its mean significance related of group 1 or group 2 or group 3 with control group . **: its mean significance related of group 1with group 2 or group 1 with group 3 . ***: its mean significance related of group 2 with group 3. ****: its mean significance related of group1 with group 2 with group 3.

Table (8) Vitamin C (mg/dL) level for gas workers (empatting and equipment) of period compared with control.

	The groups	N	Mean ± SD mg/dL	SE	95% CI		P-value
					Lower	Upper	
kers empatting of period	G1	12	0.134 ± 0.021	0.006	0.120	0.147	0.000*
							0.086****
	G2	11	0.120 ± 0.017	0.005	0.108	0.132	0.000*
							0.088**
	G3	9	0.109 ± 0.023	0.007	0.090	0..127	0.000*
							0.068**
							0.386***
Workers equipment of period	G1	5	0.200 ± 0.066	0.029	0.117	0.283	0.001*
							0.068****
	G2	12	0.124 ± 0.020	0.005	0.111	0.137	0.000*
							0.089**
	G3	11	0.110 ± 0.023	0.006	0.095	0.126	0.000*
							0.016**
							0.175***
	Control	42	0.804 ± 0.370	0.057	0.540	1.920	--------

C: workers of period groups (empatting and equipment) :-

*: its mean significance related of group 1 or group 2 or group 3 with control group . **: its mean significance related of group 1with group 2 or group 1with group 3 . ***: its mean significance related of group 2 with group 3. ****: its mean significance related of group1 with group 2 with group3.

Conclusions:

1-Oxidative stress is a common consequence of exposure to different types of environmental pollutants during work times in various types of professions in Iraq.

2-Workers with different duration of exposure to LPG demonstrated significant differences in peroxynitrite,MDA In conclusion, the changes in lipid peroxidation this indicator for the impact of duration of exposure for LPG workers.

3-The significantly increased in MDA and Peroxynitrite level and decreased in vitamin E and vitamin C.

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