Chelonian Conservation And Biology

Vol. 19No.1 (2024) | https://www.acgpublishing.com/ | ISSN - 1071-8443

DOI:doi.org/10.18011/2024.01(1). 1004-1012

STUDYING THE EFFECT OF BENZENE ON THE LEVEL OF A NUMBER OF VARIABLES AND PERCENTAGE OF FATTY ACIDS FOR WORKERS IN GAS STATIONS IN MOSUL

Noor A. Abd Al-Jabbar[1], Reem N. Alsawaf[2], Ebtehag Z. Sulyman[3]

[1&3]Department of Chemistry, Education College for girls, University of Mosul, Mosul, Iraq,

[2]Ministry of Education / Nineveh Education Directorate

noor.22gep10@student.uomosul.edu.iq[1],reemalsawaf86@gmail.com[2], ebthalim@uomosul.edu.iq[3]

***Corresponding Author: Noor A. Abd Al-Jabbar**

*Department of Chemistry, Education College for girls, University of Mosul, Mosul, Iraq, noor.22gep10@student.uomosul.edu.iq

Abstract

This study included the estimation of a number of biochemical variables related to antioxidants and the percentage of fatty acids. Glutathione(GSH), manoaldehyde (MDA), and the total capacity of antioxidants (TAC)in the blood serum of men who work at gas stations in the city of Mosul / Iraq. The workers were selected so that they did not suffer from any apparent disease (heart disease / diabetes / blood pressure) and were exposed on a daily basis to benzene and petroleum product pollutants, and the results of these variables were compared with a group of men of the same ages who do not work in the field of petroleum products. In this study, the percentage of fatty acids of different types (saturated and unsaturated) in the three parts of blood serum was measured: triglycerides(TG), phospholipids(PL), and cholesterol esters(CE). The results showed that there were significant differences in the level of biochemical variables and the percentage of fatty acids between the group of workers and the control group, which indicates the presence of an effect of gasoline and oil pollutants on the health of workers in this field.

Keywords: GSH, MDA,TAC, gasoline, fatty acids , gas stations

Introduction

Gas stations are a fundamental source of air pollution in urban areas and modern. One of the source polluting compound is benzene , a volatile chemical compound that with other compounds constitutes gasoline (**Abdulameer, A. H., & Hussein, S. Z.,** ***2023***) .It exposure related with provide promising disruption of mitochondria pathway, system biology biomarker for risk estimation of benzene encourage hematotoxicity humans (**Ali, etal, 2022**) .people are exposed to gasoline vapors though recharging and fueling at gas station .but gas station workers are at upper risk due to their work environmental (**A mani Q.Alhadithi δ Faiza Kadhim ;2023**).As a result, seeing on gas station workers who are exposed to gasoline on a regular basis appeared required(**Lewné, M etal , 2006**) . Benzene is material that is extensively found in the environment .The main bases of benzene in the environment are industrialized operation .Releases from oil burning benzene remaining and coal and motor vehicle exhaust and vaporization from gasoline services and storage operations can all raise benzene levels in the air(**Wilbur, S etal , 2008**).

Fatty acids contain a linear chain of even numbers of carbon atoms with hydrogen atoms along the chain at one end of the chain and a COOH carboxyl group at the other end. It is the carboxyl group that turns it into a carboxylic acid. If the carbon-carbon bonds are simple, the acid is saturated; If any of the bonds are double or triple, the acid is unsaturated (britanica). Lipid peroxidation of polyunsaturated fatty acids (PUFAs) occurs when oxidants, such as free radicals or non-radical species, remove one of the unstable hydrogen atoms from the methylene groups in the dual position.. This is the reason behind the generation of lipid peroxidation radicals and hydroperoxides (N.K. Dewi and Ayuniastuti, 2019). A previous study suggests that contact with benzene induces disturbances in metabolic levels of the FAO pathway for fatty acid oxidation. Therefore, FAO may be a major pathway related to benzene septicemia. But the specific effect of benzene on the enzyme in the FAO pathway is still unclear (Sun, R et al 2016).

Glutathione (GSH) is minor antioxidant that avoids chain reactions by accumulating free radicals and reactive oxygen species shows a function in tissue oxidative stress preventing (**Sidebang, P etal,2023).**

Malondialdehyde (MDA) is a biomarker of lipid peroxidation and one of the oxidative harm biomarkers in the human body it is poisonous producing OS to injury the cell membrane. Thus, MDA should be compact if it has attain it is maximum level.(**H. Winarzi etal ,2012***)* .

Blood contains many antioxidant molecules that inhibit and/or prevent harmful free radical reactions (Fartosy, A. J etal 2017). Plasma antioxidant concentrations can be measured separately in a research laboratory, but these measurements are time-consuming, labor-intensive, and expensive. Because the antioxidant effects of plasma antioxidant components are additive, measurement of total antioxidant capacity (TAC) reflects plasma antioxidant status. We estimated total plasma antioxidant status using TAC (Benzie IF 1996; Benzie IF 1999).

In the present study, we observe the effects of benzene on key enzyme in the fatty acid pathway, as well as its effect on total anti-oxidant (TAC), (GSH) and lipid peroxidation (MDA) .

Material and methods:

Blood serum samples were collected from workers at gas stations = For people who do not suffer from any apparent diseases (diabetes, blood pressure, heart disease) (57) samples, as well as from people who do not works at gas stations (37)samples.

It was collected (5 ml) of blood was drawn after fasting (12-14) hours, and the sample was placed in sterile plastic tubes free from (EDTA) and placed in a water bath at 37 °C. Then the blood serum was separated using a centrifuge at (3000 xg) for 10 minutes, and then the serum was taken and placed freeze at) -18°C (until measurements .

Extraction fatty acid

Taken serum (1ml) and added (2 ml)of methanol and (4 ml) of chloroform were added to it in a ratio (2:1) with shaking, then filtered. The filtrate was taken and its volume was reduced to (0.5 ml) using nitrogen gas. Then the samples were passed on a thin layer chromatography(TLC) plate. At dimension (20*20 cm) for 45 minutes, after that, the separated fat spots were highlighted using 2,7 dichlorofluronces dye, and the phospholipid fat, triglyceride, and cholesterol ester spots were scraped . Then, the process of re-esterification of the separated fats was performed using a solution BF_3 dissolved in methanol at a concentration of 14%((**Ma et al** ;**1995)**). The resulting samples were stored after esterification sealed tubes until conduct fatty acid analysis using HPLC.

HPLC condetion:

The HPLC system used in this study contains two SYKAM pumps, a German solvent delivery system, and a fluorescence spectrophotometer detector operating at excitation and emission wavelengths of 265 and 315 nm, respectively. The analytical column was a C18-ODS shim pack (250 mm * 4.6 mm). The gradient conditions for acetonitrile were as follows: A: (85-15%) from 0 to 4 minutes, B: (87-13%) from 5 to 8 minutes, and C:(97-3%) from 9 to 14 minutes. The vertical oven temperature was set at 50 °C and the mobile phase was filtered, degassed, and pumped at a flow rate of 1.5 ml/min. (Majnooni, M, et al; 2016).

Measurement of malondialdehyde (MDA) level

The malondialdehyde levels in the serum were estimated, which reflects the amount of lipid peroxides, as it is a final product of lipid oxidation. The method is based on the reaction between malondialdehyde and thiobarbituric acid (TBA) which takes place in an acidic medium and which forms a coloured complex that absorbs light at a wavelength of (532nm). (Muslih et al 2002).

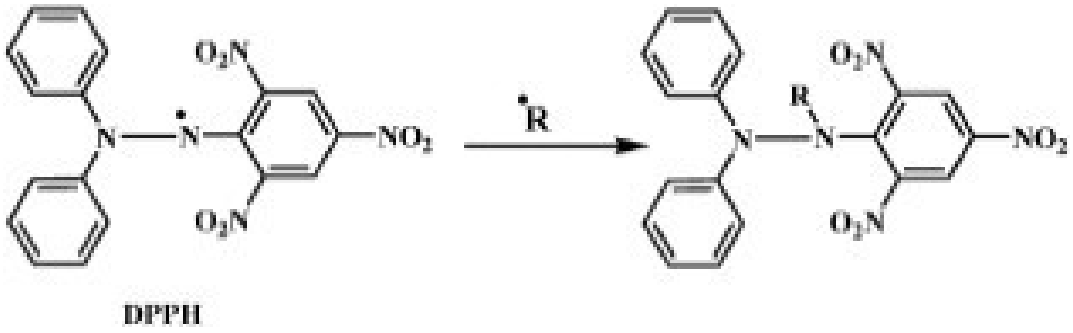
Measurement of glutathione (GSH) level

The glutathione level was measured according method (Sedlak *et al* 1986) contains use Elman's solution involves (5,5 – di thio bis-2-nitro benzoic acid)(DNTB) , it interacts e reagent with glutathione to produce yellow complex the absorbance measure at 412 nm.

Determination Total Antioxidant Capacity (TAC):

Basic principle

The total antioxidant capacity (TAC) was measured according to the (DPPH) method, which is the method used in comparison with vitamin C, as shown in the equation (1) (Nimse & Pal; 2015)



DPPH

Equation (1): DPPH Assay

(Nimse & Pal; 2015)

The 2, 2'- diphenyl -1- picrylhydrazyl radical was dissolved in methanol (400µg/ml) (Okunade, 2002). To make a standard solution of (vitamin C) a sample of (0.5) gm of vitamin (C) such was taken and mixed with a (100 ml) of methanol and distilled water (1:4 ratio v:v)The concentration of the stock solution (5000 ppm) was a gained using the dilution law, and from which other concentrations were prepared (31.25, 62.60, 100, 200, 400, and 600 ppm) from vitamin (C) and a sample of(Vaidyaratnam, 2002) ,The mixture was shaken vigorously and allowed to stand at room temperature. for 30 minutes then, after which its absorbance was measured at (517) nm. using a spectrophotometer (Ahmed ;2013), (Koleva; 2002)

Calculation

The percentage DPPH, was calculated using the following equation:

DPPH % = (A0 –A1/A0) x100

Where:

A0 : absorbance blank

A1 : absorbance sample

Results:

The results of this study showed a significant increase in the level of (MDA) and a significant decrease in the level of (GSH), as well as for (TAC), as shown in Table No. (4). The results also showed that there were significant differences, both increase and decrease, in the percentage of fatty acids of various types, saturated. And unsaturated in the three parts of blood serum, when comparing the group of workers at gas stations with people who do not work in the field of filling fuel or petroleum derivatives, as shown in Tables (1, 2, and 3).

Table (1):Fatty acid ratio in phospholipid part

Fatty acid	Mean ±S.D Non-workers	Mean ±S.D Workers	P value ≤ 0.05
Lenolinic %	4.615±0.664	6.883±0.308	0.000
Oleic%	1.775±0.483	3.455±0.099	0.000
Linoleic%	3.5930±0.554	5.0490±0.123	0.000
Palmetic %	0.1840±0.316	0.9050±0.776	0.000
Stearic%	0.0420±0.161	0.1140±0.135	0.000

Table(2):Fatty acid ratio in triglyceride part

Fatty acid	Mean ±S.D Non-workers	Mean ±S.D Workers	P value ≤ 0.05
Lenolinic %	4.4800 ± 0.6413	6.9300 ±0.2820	0.000
Oleic%	1.7256±0.4416	3.2418 ± 0.738	0.000
Linoleic%	3.5290 ± 0.6012	5.0340 ± 0.2190	0.000
Palmetic %	0.1780 ± 0.329	0.900 ±0.6566	0.000
Stearic%	0.0360 ± 0.0143	0.1080 ± 0.007	0.000

Table(3):Fatty acid ratio in cholesterol ester part

Fatty acid	Mean ±S.D Non- workers	Mean ±S.D Workers	P value ≤ 0.05
Lenolinic %	4.455 ± 0.646	6.928 ± 0.207	0.000
Oleic%	1.994± 0.632	3.4630 ± 0.0600	0.000
Lenoleic%	3.9150 ± 0.7186	4.799 ±0.319	0.002
Palmetic %	0.1750 ± 0.0208	0.900 ± 0.0656	0.000
Stearic%	0.0410 ± 0.0207	0.118 ± 0.0154	0.000

Table (4) : biochemical parameters

Parameters	**Mean ±S.D Non-workers**	**Mean ±S.D Workers**	**P value ≤ 0.05**
Glutathione (GSH) µmol/L	4.229 ± 1.107	2.087 ± 1.115	0.000
Malondialdehyde(MDA) µmol/L	0.868 ± 0.038	1.057 ± 0.239	0.020
Total Antioxidant Capacity (TAC) (ppm)	15.892 ± 0.429	14.2510 ± 0.211	0.000

Discussion:

Studies have shown that there is a significant effect of benzene on the function of various organelles, especially mitochondria and the process of oxidative stress. Recent research has proven that there is a significant difference in the different paths of fatty acid metabolism, and major changes occur in the metabolism of fatty acids due to the effect of benzene on them and on many different paths, especially the carnitine path, which is considered one of the Important pathways in transporting fatty acids to and from the mitochondria due to the importance of these acids in the formation of stem cells and controlling the automatic renewal of these cells (**Ito, K., & Suda, T. 2014**), ecent studies have shown that exposure to benzene on a continuous basis causes blood poisoning through the significant difference in the process of metabolism and metabolism of fatty acids in the mitochondria and the bioenergy pathways specific to those processes. These facts can be confirmed through the significant effects of benzene on the decomposition of sugars and hexaphosphate and many pathways. Mitochondria such as the pathway (BCAAS) and butanoate metabolism (**McHale, C.M. *et al.* 2011**).

Various studies, including this study, recorded a significant decrease in the level of linolenic acid, and this indicates the presence of many changes associated with the process of oxidative stress, as polyunsaturated fatty acids are highly susceptible to the processes of fat peroxidation through the attack of the oxidizing agent on a number or one of the carbon-carbon double bonds. Contained in the composition of fatty acids (**Yin, H & Porter,N. 2011**).

Some studies have shown the presence of very high levels of a number of isomers of the fatty acid hydroxylene acid, which indicates an increase in the processes of oxidative stress and the formation of free radicals (ROS) from comparing the results and their association with the effect of benzene, as these compounds resulting from the process of fat peroxidation are more easily reduced to hydroxyl acids. Interview, where the change in oxidative stress is considered a major biological response as a result of exposure to gasoline by gas station workers, and these changes in oxidative processes are significantly related to exposure to a wide range of polluted environments and increase the incidence of blood diseases. (**Rothman, N, 2020**) .

A number of studies have also shown that there is a lot of evidence that changes in the oxidation of fatty acids, including environmental changes and gene expression, especially those related to

the process of transporting fatty acids and beta-oxidation on them due to the presence of benzene and continuous exposure to it. (**Siddiqui, A. *et al*. 2020).**

Because of the large increase that occurs in the formation of free radicals due to benzene, this leads to the consumption of a lot of internal antioxidants (inside the body). This is caused by the imbalance between the formation of free radicals and antioxidants resulting from the major imbalance in the oxidation and reduction process within oxidative stress. (**Dewi, N.**K. 2019).

The process of oxidative stress occurs as a result of a major imbalance between the number of free radicals formed and the number of antioxidants in the body, so that the number of free radicals is much greater than the number of antioxidants due to the occurrence of an excessive process of fat peroxidation, as the value of (MDA) increases due to the increase in fat peroxidation, as The value of (MDA) is considered a positive and vital indicator that expresses the process of oxidative stress (**Kunwar A and Priyadarsini 2011)**.

Internal antioxidants work to help the body resist the process of oxidative stress, but within limits. When the number of free radicals increases significantly outside the range of resistance to internal antioxidants, this requires the presence of external antioxidants that come from food, by extracting a number of these antioxidants from plants, especially fruits. Through certain means, external and internal antioxidants participate in resisting the free radicals formed, which leads to reducing the process of oxidative stress. (**Owagboriaye F O,2018**).

The results of this study indicated a significant decrease in the total capacity of free radicals (TAC) among workers in fuel filling stations with an increase in the time period of exposure to gasoline, as exposure to heavy metals present as pollutants in gasoline leads to an increase in (ROS) and thus a decrease in the amount of (TAC). (**Aida AH, 2013**). Other studies have found that increasing the time period of exposure to benzene causes an increase in pollution, an increase in the formation of ROS, and a significant decrease in enzymatic and non-enzymatic antioxidants, in order to reduce the oxidative stress resulting from this process. (**Uzma N.,2010**).

References:

1. Lewné, M., Nise, G., Lind, M. L., & Gustavsson, P. (2006). Exposure to particles and nitrogen dioxide among taxi, bus and lorry drivers. *International archives of occupational and environmental health*, *79*, 220-226.
2. Amani Q. Alhadithi , Faiza Kadhim (2023). Detecting the concentration of some Trace Elements in Blood Samples of Fuel Station Workers .
3. Wilbur, S., Wohlers, D., Paikoff, S., Keith, L. S., & Faroon, O. (2008). ATSDR evaluation of health effects of benzene and relevance to public health. *Toxicology and industrial health*, *24*(5- 6), 263-398.
4. Rothman, N., Vermeulen, R., Zhang, L., Hu, W., Yin, S., Rappaport, S. M., ... & Walker, D. I. (2021). Metabolome-wide association study of occupational exposure to benzene. *Carcinogenesis*, *42*(11), 1326-1336.

5. Abdulameer, A. H., & Hussein, S. Z. (2023). Assessment of Oxidative Stress Parameters for some of Baghdad City Fuel Stations Workers. *Iraqi Journal of Science*, 2669-2680.
6. N.K. Dewi and Ayuniastuti , ― Glutathione and malondialdehyde levels in gas station attendants of sermaramge city ― J phys Conf Ser ., *vol.132 1, no.19,pp.1-12, 2019*
7. Sun, R., Cao, M., Zhang, J., Yang, W., Wei, H., Meng, X., ... & Pu, Y. (2016). Benzene exposure alters expression of enzymes involved in fatty acid β-oxidation in male C3H/He mice. International journal of environmental research and public health, 13(11), 1068.
8. *Sidebang, P., Kusumayati, A., & Haryanto, B. (2023). Oxidative Stress Levels of Fine Particulate Matter (PM2. 5) and Urinary Glutathione of Microbus Drivers. Kesmas: Jurnal Kesehatan Masyarakat Nasional (National Public Health Journal), 18(1), 9-15.*
9. *H.* Winarzi , S. P. M . Wijayanti and APurwanto, ―Aktivitas Enzim Superoksida Dismutase , katalase , dan glutathione Peroksidase Wanita Penderita Sindrom Metabolik‖ Maj Kedokt Bandung, vol.44, no. 1,pp. 7-12, 2012
10. Fartosy, A. J., Shanan, S. K., & Awad, N. A. (2017). Biochemical Study of The Effects of Some Heavy Metals on Oxidant Antioxidant Status in Gasoline Station Workers Basra- Iraq. *International Journal of Scientific and Research Publications*, *7*(2), 83-94
11. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of ―antioxidant power‖: the FRAP assay. Anal Biochem 1996;239:70–6.
12. Benzie IF, Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol 1999;299:15–27
13. Majnooni, M. B., Mohammadi, B., Jalili, R., Babaei, A., & Bahrami, G. (2016). Determination of fatty acids by high-performance liquid chromatography and fluorescence detection using precolumn derivatization with 9-fluorenylmethyl chloroformate. *Journal of Liquid Chromatography & Related Technologies*, *39*(19-20), 877-881.
14. Ito, K., & Suda, T. (2014). Metabolic requirements for the maintenance of self-renewing stem cells. *Nature reviews Molecular cell biology*, *15*(4), 243-256.
15. Yin, H., Xu, L., & Porter, N. A. (2011). Free radical lipid peroxidation: mechanisms and analysis. *Chemical reviews*, *111*(10), 5944-5972.
16. Dewi, N. K. (2019, October). Glutathione and malondialdehyde levels in gas station attendants of Semarang city. In *Journal of Physics: Conference Series* (Vol. 1321, No. 3, p. 032039). IOP Publishing.
17. Kunwar A and Priyadarsini 2011 *Journal of Medical and Allied Sciences* 1 53
18. Aida AH., Sahar AA., Amal FG. and Sarah AB."Assessment of oxidative stress and antioxidant status among petrol stations' workers exposed to benzene in zagazig city . *Z.U.M.J.*, 19(5), pp : 446-457, 2013
19. Uzma N., Kumar B. and Hazari M. "Exposure to Benzene Induces Oxidative Stress, Alters the Immune Response and Expression of p53 in Gasoline Filling Workers" *American J. of Industrial Medicine*, 53, pp:1264–1270, 2010.

20. Muslih, R., AL-Nimer, M., and AL-Zamely, O. (2002). The level of malondialdehyde after activation with (H2O and CuSO4) and inhibition by disferoxamine myocardial infraction. National Journal of Chemistry, 5, 139-148.
21. McHale, C.M. *et al*. (2011) Global gene expression profiling of a populationexposed to a range of benzene levels. *Environ. Health Perspect.,* 119, 628–634.
22. Siddiqui, A. *et al*. (2020) A non-proliferative role of pyrimidine metabolismin cancer. *Mol. Metab.,* 35, 100962.
23. Rothman, N., Vermeulen, R., Zhang, L., Hu, W., Yin, S., Rappaport, S. M., ... & Walker, D. I. (2021). Metabolome-wide association study of occupational exposure to benzene. *Carcinogenesis*, *42*(11), 1326-1336.
24. li, A. M., Alsaqa, R., & Sultan, N. S. (2022). Study of Thermodynamic Properties of Monoclinic Sulfur (Sβ) Under High Pressure Using Three Different Equations of State for the Treatment Scabies in Dermatology. *International Journal of Thermodynamics*, *25*(2), 33-38.
25. Ma, J., Folsom, A. R., Shahar, E., & Eckfeldt, J. H. (1995). Plasma fatty acid composition as an indicator of habitual dietary fat intake in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. *The American journal of clinical nutrition*, *62* (3), 564-571.
26. Okunade, A. L. (2002). Review: Ageratum conyzoides L. *Asteraceae) Fitoterapia*, *73*, 1-16.
27. Nimse, S. B., & Pal, D. (2015). Free radicals, natural antioxidants, and their reaction mechanisms. *RSC advances*, *5* (35), 27986-28006.
28. Saleh, M. M., Khemakhem, H., Jassim, I. K., & Al-Saqa, R. H. (2024). Structure and mechanical properties of cermet Ni-Al/MSZ thick coating prepared by flame spraying technique. *Ochrona przed Korozją*.
29. Vaidyaratnam, Varier, PS, (2002) Indian Medicinal Plants- A Compendium of 500 species, I, Orient longman publishing house, Kottakkal-India, 146. 15.
30. Koleva, I. I., Van Beek, T. A., Linssen, J. P., Groot, A. D., & Evstatieva, L. N. (2012). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, *13* (1), 8-17.
31. Thomas, T. M., Enge, K. M., Suarez, E., Schueller, P., Bankovich, B., & Leone, E. H. (2023). Home Range and Habitat Selection of the Suwannee Alligator Snapping Turtle (Macrochelys suwanniensis) in the Suwannee River, Florida. Chelonian Conservation and Biology: Celebrating 25 Years as the World's Turtle and Tortoise Journal, 22(2), 146-155.
32. KAREEM, S., UONİS, M., & ALSAQA, R. (2023). High-Pressure Calibration TiN Equation of State. *International Journal of Thermodynamics*, 1-7.
33. Sedlak J. and Lindsay R.H. 1986. Analytical biochemistry.P.192.cited by Al-Zamle O.M., Al-Nimer M.S, and AL-Muslih R.K, 2001. Detection the level of level of peroxynitrite and related with antioxidant status in the serum of patients with acute myocardial infraction. Nat J Chem., 4: 625-637