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Cutaneous Toxicity of Gasoline as an Environmental Pollutant on Mice Skin: Histological and Ultrastrucural Studies

Awatef Ali[1*] --- Nawal El-Ghazaly[2] --- Samir Dekinesh[3] --- Sanaa Ahmed[4] --- Azza Sedky[5]

[1,2,3,5]Zoology department -Faculty of Science -Alexandria University-Egypt
[4]Genetic &Biotechnology institute-City of Scientific Researches-Alexandria University- Egypt

Abstract

The present investigation was undertaken to determine whether gasoline with different octane number (90 and 80) exposure in healthy Swiss albino mice might affect the skin, in order to declare more recognition on the adverse changes that might occur as a result of exposure to this pollutant.
Sixty Swiss albino mice were used and divided into 3 subgroups : control-subgroup, topically treated -subgroup with gasoline 90 and topically treated -subgroup with gasoline 80, both groups two and three treated with (0.5ml/kg B.W.) for 8 weeks.
Determination of benzene concentration and some heavy metals in both types of gasoline (90 and 80), determination of some heavy metals bioaccumulation in skin after exposure to both types of gasoline (90 and 80),light and electron microscopical studies were performed.
It was found that gasoline 80 contained more concentrations of benzene, lead , cadmium and nickel than gasoline 90. The accumulation of lead, cadmium and nickel in the skin have the following order lead > cadmium > nickel. The light microscopical examinations showed dermatitis such as epidermal hyperplasia, micro abscesses, hyperkeratosis and destruction of the dermis depending upon treatment duration from 4 to 8 weeks. After 8 weeks of topically treated mice with both gasoline type , the scanning electron microscope examination showed scales covering the mice skin and the transition electron microscope showed cytoplasmic vacuoles and mitochondrial degeneration in keratinocytes of all epidermal layers of the mice skin . Moreover, the spinosum keratinocytes of mice skin topically treated with gasoline 80 for 8 weeks had pyknotic nuclei,in addition to expansion of intercellular spaces in the stratum corneum.

Keywords: Gasoline, Mice, Skin, Bioaccumulation, Histopathology,Light and electron microscope.

Contents

** Corresponding Author*

1. Introduction

Gasoline is very light oil produced from refinement of petroleum. It is popularly known as fuel where it is used in internal combustion engines. It is also used as solvent mainly, known for its ability to dilute paints. It is used or disposed in large quantities and therefore may cause significant and potential health risks to environment and human being [1]. The major industries using benzene are those involving rubber, paint, shoes, lubricants, dyes, detergents, drugs and pesticides. However, there are environmental exposures to benzene at gasoline stations and from individuals who smoke [2].

Gasoline is a mixture of hydrocarbons including a variety of branched and unbranched aliphatic hydrocarbons as well as aromatic hydrocarbons with varied composition according to its place of origin. In general, it contains alkanes, alkenes, alcohol, ether and many additives such as benzene and lead [1]. Benzene is a common environmental contaminant found in gasoline [3].

Many adverse health effects of gasoline are due to individual chemicals in gasoline that are added in small amounts to improve car performance such as methyl tertiary butyl ether (MTBE), benzene, toluene and xylene [4].

Benzene is a toxic aromatic having a chemical formula (C_6H_6) [5]. Chronic exposure to benzene can lead to deleterious effects on many biological systems [6]. Benzene is one of the few known etiological factors for tumors at multiple sites in rats and mice [7] and acute myelogenous leukemia (AML) in humans [2].

Exposure to fuels is a significant occupational hazard for workers in fuel station. Daily exposure to fuels result in saturation of the cotton cloth, resulting in an occluded environment for repeated, long term exposure to the skin during the typical 8-hours working [8].Prolonged or repeated contact with gasoline and other petroleum solvents may cause defeating and fissuring in the skin with resultant dermatitis [9].Skin exposed to JP-8 jet fuel showed increase in number of granulocytes in mice and epidermal thickness, micro abscess and edema in pig [10].

In this study, two kinds of motor gasoline (vehicle fuel in Egypt), one gasoline 90 and the other gasoline 80, will be used in order to find out if the hazards produced by gasoline 90 are less or more than those produced by gasoline 80 to declare more recognition on the adverse changes that may occur as a result of exposure to this pollutant. The topical exposure was chosen because the skin irritation by chemicals as fuels can be a major problem in the work place and the home.

2. Materials and Methods

2.1. Materials

The male mice were 10 weeks old, weighing 25-30 gm. each, and reared in usual type of metal cages (30 cm length, 20 cm width and 15 cm high) with wood shaving as substrate. Animals were allowed to acclimatize, one week before the initiation of the experiments, under normal laboratory conditions. They were allowed free access of a standard balanced laboratory diet (wheat, milk and carrot) and tap water.

The studies on the skin in topical exposure groups were done by shaving area of the back skin (3×4 cm). Animals were left at least 24 h after hair removal to ensure that no irritation occurred from the hair removal process. Gasoline liquid (0.5 ml/kg B.W.) was dropped (0.1ml/mouse/day) onto the shaved area. To minimize the exposure of animal by inhalation, treatment was performed with cold gasoline (4ºC). Each animal was treated slowly to avoid any significant spillover and keep separately from each other until gasoline had dried.

Sixty mice were used and were divided into three subgroups, each of 20 mice: Ga (control subgroup): daily locally exposed to drop of tap water on its shaved back skin, Gb (treated subgroup): daily locally exposed to drop of gasoline (octane 90) on its shaved back skin and Gc (treated subgroup): daily locally exposed to drop of gasoline (octane 80) on its shaved back skin .The experiment extended for 8 weeks.

2.2. Methods

2.2.1. Biochemical Studies

2.2.1.1. Determination of Benzene Content in Both Types of Gasoline (90 and 80):

Gas chromatography is equipped with split flow of 100 ml/ injector and silica capillary column; Thermo TR-35 MS (30m,0.25mm,0.25µm) with 35% phenyl polysilphenylenesiloxane. Nitrogen was used as carrier gas at 0.5 ml/ min. The oven temperature program was 80º C (hold for 2 minutes), ramped at 5º C/min to 150º C, hold for 5 minutes then ramped at 10º C/min to 200º C maintaining about 4º C. Temperatures of the injection, and the flame ionizing detector were maintained at 200 and 250º C, respectively.

Determination of some heavy metals in both types of gasoline (90 and 80) : According to Ahmed, et al. [11] .

2.2.1.2. Determination of Bioaccumulation of Some Heavy Metals in Skin After Exposure to Both Types of Gasoline (90 and 80)

After 8 weeks skin was excised from topical exposure group. The concentration of lead, cadmium and nickel were determined by using electron dispersive X-ray apparatus attached to scanning electron microscope (Leo – UIF: Leo438VP) of Faculty of Science, Alexandria University.

2.2.2. Microscopical Studies

For microscopical observations, mice were randomly chosen and scarified after 4 and 8 weeks of experiments. The skin from control and treated subgroups was excised and three small portions of it were taken. The first portion fixed at room temperature overnight in 10% formalin solution then process to be stained routinely with Haematoxylin and Eosin according to Bancroft and Gamble [12] (after 4,8 weeks) for light microscope. The second portion processed for scanning electron microscopic study and examined using Leo-UIF: Leo 438 VP scanning electron microscope of the Faculty of Science, Alexandria University (after 8 weeks).The third portion processed for transmission electron microscopic observation and were examined using Joel 100 CX transmission electron microscope of the Faculty of Science, Alexandria University (after 8 weeks).

3. Results

3.1. Determination of Benzene Content and Some Heavy Metals in Both Types of Gasoline (90 and 80)

The concentration of benzene in both types of gasoline 90 and 80 was 0.897% and 1.17% volume/volume (v/v) respectively (Table 1). In addition, the concentration of lead, cadmium and nickel in gasoline 90 were 0.241, 0.013 and 0.009mg/L respectively while in gasoline 80 they were 0.399, 0.098 and 0.023mg/L respectively. From table (1), it could be noticed that the lead had the highest concentration followed by cadmium then nickel in both types of gasoline 90 and 80.

3.2. Bioaccumulation of Some Heavy Metals in the Skin after Exposure to Both Types of Gasoline (90 and 80)

3.2.1. Lead Concentration

From table (2) the mean value of lead concentration in the mouse skin topically treated with gasoline 90 after 4 weeks (3.87 ± 0.30) and after 8 weeks (4.57 ± 0.45) which is higher than control mice (0.13 ± 0.13) .The mean value of lead concentration in mouse skin topically treated with gasoline 80 for 4 weeks (6.0 ± 0.97) and for 8 weeks (10.07 ± 0.71) which is higher than control mice (0.13 ± 0.13% total) so, there were significant difference between the control mice and those topically treated with gasoline 90 or 80 for 4 and 8 weeks ($F = 35.035$, $p = 0.001$).

3.2.2. Cadmium Concentration

From table (2) the mean value of cadmium concentration in the mouse skin topically treated with gasoline 90 after 4 weeks (1.40±0.26)and for 8 weeks (2.77 ± 0.73) which is higher than control mice (0.20 ± 0.06%) . The mean value of cadmium concentration in mouse skin topically treated with gasoline 80 for 4 weeks (3.33±0.34) and for 8 weeks (3.80 ± 0.57%) which is higher than control mice (0.20 ± 0.06%)so, there were significant difference between the control mice and those topically treated with gasoline 90 or 80 for 4 and 8 weeks ($F = 35.035$, $p = 0.001$).Also, there was no significant difference between the mean values of cadmium concentration in mouse skin topically exposed to both types of gasoline .

3.2.3.Nickel Concentration

From table (2) the mean value of nickel concentration in the mouse skin topically treated with gasoline 90 after 4 weeks (0.43 ± 0.07) and for 8 weeks (0.87 ± 0.09% total) which is higher than control mice (0.10 ± 0.06% total). The mean value of nickel concentration in the mouse skin topically treated with gasoline 80 after 4 weeks (1.50 ± 0.25) and for 8 weeks (1.67 ± 0.44) which is higher than control mice (0.10 ± 0.06% total)so, there were significant difference between the control mice and those topically treated with gasoline 90 or 80 for 4 and 8 weeks ($F= 8.26$, $P = 0.003$).

3.3. Microscopical Results

3.3.1. Light Microscopical Observations:

3.3.1.1. Subgroup Topically Exposed to Gasoline 90.

After 4 weeks, the mouse skin displayed absence of dermal papillae with increased number of hair follicles (Figure1).The epidermis showed hyperkeratosis, detached thick keratin layer, thickness of dark stratum granulosum and stratum basal with depicting paranuclear vacuolated cells and pyknotic nuclei. The epidermis was followed by degenerated dermis (inserted part). After 8 weeks, the normal architecture of skin disappeared; keratin layer was detached, the dermis showed increased number of hair follicles and degenerated areas (Figure 2).

3.3.1.2. Subgroup Topically Exposed to Gasoline 80.

After 4 weeks, the mouse skin revealed architectural alterations as thin stratum corneum, epidermal hyperplasia and disorganized lytic dermis filled with hair follicles and sebaceous gland. Sub corneal abscesses appeared as round vesicles filled with blood cells (Figure 3). After 8 weeks ,absence of stratum corneum, epidermis with thin and thick area ,thick dermis with alternating dense and loose areas (Figure 4) obvious increase in hair follicles were detected (inserted part).

3.3.2. Electron Microscopical Observations:

3.3.2.1. Scanning Electron Microscopical Observations

3.3.2.1.1. Subgroup Topically Exposed to Gasoline 90 For 8 Weeks

Revealed presence of thick destructed hair shaft with broken hair sheath and increased hair number. Few spindle and polygonal scales covering the dissociated skin surface (Figure 5).

3.3.2.1.2. Subgroup Topically Exposed to Gasoline 80 for 8 Weeks

Showed many thick destructed hair shafts with broken hair sheath and stratum corneum units separation. Abundant large spindle and polygonal scales detached from skin surface (Figure 6).

3.3.2.2. Transmission Electron Microscopical Observations

3.3.2.2.1. Subgroup Topically Exposed to Gasoline 90 for 8 Weeks

Revealed hyperplasia of both stratum spinosum with flat nuclei and stratum granulosum with keratinohyaline granules (Figure 7). There was degeneration of the basal keratinocytes near the dermis as irregular nucleus with chromatin condensation, dilated nuclear envelope and cytoplasmic invagination. Dark cytoplasm contained

accumulation of tonofilaments at perinuclear area, many cytoplasmic vacuoles and degenerated mitochondria (Figure 8).In the spinosum keratinocyte membrane bound vesicles are frequently seen in the cytoplasm, accumulated tonofilaments, round keratinohyaline granules and irregular nucleus with many nucleoli. The nucleoli are usually located close to the nuclear envelope. Degenerated mitochondria were also seen (Figure 9).Also; granulosum keratinocytes had patches of electron dense granules, short tonofilaments, irregular nucleus with dilated nuclear envelope and wide pores. The stratum corneum appeared as thick and disorganized parallel lamellae with many vacuoles (Figure 10).

3.3.2.2.2. Subgroup of Mice Topically Exposed to Gasoline 80 or 8 Weeks:

Showed basal keratinocyte with pale lytic nucleus, dense nucleolus, cytoplasmic vacuole, and decreased amount of tonofilaments, wide desmosomal junction, degenerated mitochondria and many free ribosomes (Figure 11). There was necrotic spinosum keratinocyte with nucleus undergoing pyknosis characterized by irregular contour and dominant heterochromatin .The cytoplasm lost its tonofilaments and had few free ribosomes (Figure 12). The stratum spinosum appeared with different alterations including keratinocyte with lytic pale nucleus had clear nucleolus and its cytoplasm had peripheral keratin tonofilaments. In addition necrotic spinosum keratinocyte with pyknotic nucleus and its cytoplasm with distributed keratin tonofilaments (Figure 13). Also, some spinosum keratinocytes appeared completely degenerated with karyolytic nuclei that completely lost heterochromatin in the degenerated cytoplasm. The stratum granulosum had degenerated keratinohyaline patches. The thick stratum corneum appeared destructed with expansion of intercellular space (Figure 14).

4. Discussion

Motor vehicle gasoline fuels are very complex mixtures containing a number of aliphatic hydrocarbons (C_3 to C_{12}) as heptane , aromatic hydrocarbons as benzene , and low molecular weight oxygenated species ,such as hydroxyl group, as well as number of heavy metals . The concentration of benzene which is the most prevalent aromatic hydrocarbons in gasoline, benzene, is of interest because of their use as octane extenders and also because of potential adverse health effects from exposure of individuals to their vapors [13].

European community laws specify a maximum tolerated level for the benzene in gasoline generally of 1% (V/V) [14]. Accordingly to the present study , benzene in gasoline 90 (0.897%) is within the allowed limit while benzene in gasoline 80 (1.17%) is greater than the allowed limit. Leaded gasoline (8191) contains the same amount of benzene as gasoline 90 (0.8%) while leaded gasoline (8129) contains the same amount of benzene as gasoline 80 [15]. Also, Pavlova and Ivanova [16] found that the amount of benzene in gasoline (Ag2H, 15.05.2002) was 0.8% while the amount of benzene in gasoline (Ag2H, 20.05.2002) was 1.1%.

Anthropogenic activities continuously increase the amount of the heavy metals in the environment. The occurrence of heavy metals in excess of natural loads caused heavy metal pollution which is growing at an alarming rate and has become an important worldwide problem. As heavy metals cannot be degraded, they are deposited in different organs of the body where their bioaccumulation resulting in health risks [17]. Putte and Part [18] stated that when animal is exposed to elevated levels of metals; it can absorb the bioavailable metals directly from the environment via the skin. Metals are then transported by blood stream which brings them to contact with the various tissues. Yousafzai, et al. [19] interpreted the high concentration of metals in the skin that the skin adsorbed the metals on its surface firstly followed by their absorption in other tissue by various mechanisms.

Vinodhini and Narayanan [20] found that lead bioaccumulated in the different organs more than cadmium and nickel with agree with the present results where the concentrations of Pb>Cd>Ni in the skin.

The toxicity of lead may be due to that lead is a divalent cation, and it binds strongly to sulfhydral group on proteins. Many of lead's toxic properties are due to its ability to mimic or complete with calcium. At picomolar concentrations, lead competes successfully with calcium for binding sites on cerebellar phosphokinase C and thereby affects neuronal signaling. It inhibits calcium entry into cells. Lead has binary impact on neurotransmitter release: spontaneous neurotransmitter release is enhanced, whereas stimulated release is inhibited [21]. Holtzman, et al. [22] stated that lead is picked up by mitochondria and produces swelling and distruction of mitochondrial cristae. Uncoupled energy metabolism, inhibited cellular respiration, and altered calcium kinetics follow. In addition, Mestek, et al. [23] reported that lead administered to laboratory rats in drinking water (0.1-0.8%) as lead acetate solution tends to accumulation in the skin.

The previous studies are in concomitant with the studies of some authors in fish tissue as Singh, et al. [24] who revealed that skin showed elevated levels of bioaccumulation of metals in comparison to muscle tissue, Naghshbandi, et al. [25] reported that crayfish exposed to intermediate concentration of lead nitrate for periods up to 3 weeks exhibited amount of heavy metal in the tissue of crayfish and Yousafzai, et al. [19] who found that skin of common crap fish also accumulated heavy metals more than the liver.

These data suggested that the primary target organ of the heavy metals is the skin. This may be due to the direct contact between skin and environmental pollutants in the case of topical exposure.

As concerning the cadmium toxicity,Smirjakova, et al. [26] pointed out that cadmium represents a serious industrial and environmental pollutant. It is a relatively volatile element not essential to plants, animals and human and its presence is unwonted and harmful.Cadmium is well known for its various advers effects e.g., influence on mitochondrial functions. Enhancement of lipid peroxidation and DNA chain break [27]. The oxidative damage of tissues and DNA is considered to be an early manifestation of cadmium toxicity and carcinogenicity. The oxidative effect of cadmium is indirect and is based mainly on the inactivation of thiol groups in critical molecules, inhibiting antioxidant defenses and DNA repair mechanisms [28].

Generally, toxic metals as lead and cadmium act as catalysts in the oxidative reactions of biological macromolecules therefore the toxicities associated with these metals might be due to oxidative tissue damage. Redox inactive metals, such as lead and cadmium deplete cells major antioxidants, particularly thiol containing antioxidants and enzymes. Redox-inactive metals may cause an increase in production of reactive oxygen species (ROS) such as

hydroxyl radical ($HO^{\cdot}$), superoxide radical ($O_2^{\bullet-}$) or hydrogen peroxide (H_2O_2). Enhanced generation of ROS can over cells intrinsic antioxidant defenses, and result in a condition known as "oxidative stress". Cells under oxidative stress display various dysfunctions due to lesions caused by ROS to lipid, proteins and DNA. Consequently, it is suggested that metal-induced oxidative stress in cells can be partially responsible for the toxic effect of heavy metals [29].

The last heavy metals studied in the present work are nickel which is defined by Scott-Fordsmand [30] as nutritionally essential trace metal for several animal species, micro-organisms and plants. It is widely distributed in the environment (air, water, soil and biological material) being released from both natural sources and anthropogenic activity. It finds its way into the ambient air as a result of the combustion of cool, diesel oil and fuel oil . Many effects of nickel are due to interference with the metabolism of essential metals such as Fe (II), Zn (II) , Cu (II), Mn (II) and Ca (II) or Mg (II) which can suppress or modify the toxic and carcinogenic effects of nickel. The toxic functions of nickel probably result primarily from its ability to replace other metal ions in enzymes and proteins or to bind to cellular compounds containing O-, S- and N-atoms such as enzymes and nucleic acids causing their inhibition.

The microscopical examination of organs has been used as a biomarker to evaluate the toxicity of various pollutants [31]. Kimura and Doi [32] reported that after one month of daily topical treatment with 0.1% solution of trans-retinoic acid of dorsal skin of hairless dogs, epidermal thickness occurred. Necrotic changes in the dermis were also found. These alterations were elucidated by other workers as Monteiro-Riviere, et al. [10] who reported that single dose ($67\mu l/cm^2$) dermal exposure in pigs to Jet A, JP-8 and JP-8+100 Jet fuels caused epidermal thickness .In addition, skin taken from 7-days JP-8 treated rats displayed characteristic thickened epidermis [33]. Batawy [34] showed that dermal application of undiluted gasoline fuel to the backs of mice resulted in epidermal thickness and hyperkeratosis with agree with the present results detected after exposure to gasoline 80 for 8 weeks which might stimulated a hyper proliferative epidermis that contributes to the progression of the lesion to stratum corneum.

The detection of micro abscesses was also showed by Monteiro-Riviere, et al. [10] and Muhammed, et al. [35] who studied the effects of dermal exposure to JP-8 fuel. This could observed after exposure to gasoline 80 for 4 weeks in the present work .It could be suggested that these alterations in the epidermal layers are due to the benzene and heavy metals which are more concentrated in gasoline 80 than in gasoline 90 and therefore could explain the development of the hyper proliferative state and the progression of the sub corneal lesion into the stratum corneum that was observed in the histology of the skin exposed to gasoline 80

In addition to the previous observations, there was degeneration of mitochondria developed in basal keratinocytes of skin of mice topically treated with both types of gasoline (90 and 80) for 8 weeks with agree with King and Monteiro-Riviere [36] and Monteiro-Riviere, et al. [37] who reported the presence of mitochondrial change in stratum basal after 2- chloroethyl methyl sulfide and jet fuel on the pig skin.

The spinosum keratinocytes of mice skin topically treated with gasoline 80 for 8 weeks had pyknotic nuclei and a definite thickening of the stratum granulosum, this agrees with the finding of Toś-Luty, et al. [38] in skin of rats exposed to carbonyl.

The cellular damage in the skin caused by gasoline is histopathologically characterized by expansion of intercellular space in the stratum corneum of mice treated with gasoline 80 for 8 weeks. Also, Monteiro-Riviere, et al. [37] found degradation of desmosomes and focal areas of intercellular disruption, loss of cohesion between layers.

It can be conclude that, gasoline 90 and 80 exposure must be considered potentially toxic where they had hazard impacts on mice skin, thus creating a serious threat to the life of human beings. So, exposure to gasoline should be minimized to the maximal possible extent, i.e. the period of work must be shorter and workers must use gloves to prevent contact with skin.

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References

[1] S. Isabelle, R. Mauna, and M. Rob, "The lead research group of the pan – American health organization. Lead exposure in Latin American and the caribbean EMV," *Health Perspective,* vol. 105, pp. 398 – 404, 1997.

[2] A. K. Bauer, B. Faiola, D. J. R. Abernethy, L. J. Pluta, N. A. Wong, F. J. Gonzalez, B. E. Butterworth, S. J. Borghoff, J. I. Everitt, and L. Recio, "Male mice deficient in microsomal epoxide hydrolase are not susceptible to benzene-induced toxicity," *Toxicological Science,* vol. 72, pp. 201-209, 2003.

[3] D. J. Abernethy, E. V. Kleymenova, J. Rose, L. Recio, and B. Faiola, "Human CD34+ Hematopoietic progenitor cells are sensitive targets for toxicity induced by 1,4-benzoquinone," *Toxicological Sciences,* vol. 79, pp. 82-89, 2004.

[4] S. S. Elzayab, "Petroleum and petrochemical industries. King sood university print, EL Reyad. Ercal, N.; Gurer-orhan, H. and Aykin-Burns, N. (2001). Toxic metals and oxidative stress part I: Mechanisms involved in metal-induced oxidative damage," *Current Topics in Medicinal Chemistry,* vol. 1, pp. 529-539, 2002.

[5] H. S. Ozturk, M. Kavutcu, and M. Kacmaz, *Curr. Med. Res. Opnin.,* vol. 14, pp. 47-52, 1997.

[6] N. Uzma, B. S. Kumar, and M. A. Hazari, "Exposure to benzene induces oxidative stress, alters and immune response and expression of p53 in gasoline filling workers," *American Journal of Industrial Medicine,* vol. 53, pp. 1264-1270, 2010.

[7] T. A. Mac Donald, K. Y. O Connell, and S. M. Rappaport, "Comparison of protein adducts of benzene oxide and benzoquinone in the blood and bone marrow of rats and mice exposed to (14C/ 13C6) benzene," *Cancer Research,* vol. 54, pp. 4907 – 4914.15, 1994.

[8] D. Allen, J. Riviere, and N. Monterio – Riviere, "Analysis of interleukin – 8 release from normal human epidermal keratioincytes exposed to aliphatic hydrocarbons to cell cultures via complexation with a - Cyclodextrin," *Toxical Im Via Vitro,* vol. 15, pp. 663 – 9, 2001.

[9] A. S. Susten, B. L. Dames, J. R. Bug, and R. W. Niemeier, "Percutaneous pevetration of benzene in hairless mice an estimate of dermal absorption during tirebulding opemtions," *Am J. Ind. Med.,* vol. 7, pp. 323 – 335, 1985.

[10] N. Monteiro-Riviere, A. Imman, and J. Reviere, "Effects of short-term high-dose and low-dose dermal exposure to Jet A, JP-8 and JP-8+100 Jet fuels," *Journal of Applied Toxicology,* vol. 21, pp. 485-494, 2001.

[11] N. Ahmed, L. Alikhan, and S. Sattar, "Protentiomentric stripping analysis of heavy metals in gasoline and dust particulate," *Joen-Chem. Soc. Pak.,* vol. 12, pp. 74-76, 1991.

[12] D. Bancroft and M. Gamble, *The theory and practice of histological technique,* 5th ed.: Churchil Living Stone, 2002.

[13] S. K. Poole, K. G. Furton, and C. F. Poole, "Determination of benzene and toluene in gasoline by gas chomotography using a liquid organic salt column," *J. Chromatogr. Sci.*, vol. 26, pp. 67-81, 1988.

[14] Directive, "Directive 98/70/Ec of the European Parliament and of The Council of 13 October 1998 relating to the quality of petrol and diesel fuels and amending Council Directive 93/12/EEC," 1998.

[15] L. Zoccolillo, M. Alessandrelli, and Felli, "Simultaneous determination of benzene and total aromatic fraction of gasoline by HPLC-DAD," *Chromatographia*, vol. 54, pp. 659-663, 2001.

[16] P. Pavlova and R. Ivanova, "GC methods for quantitative determination of benzene in gasoline," *Acta Chromotographica*, vol. 13, pp. 215-225, 2003.

[17] H. Agah, M. Leermakers, M. Elskens, S. Fatemi, and W. Boeyens, "Accumulation of trace metals in the muscles and liver tissues of five fish species from the Persion Gulf," *Environ. Monit. Assess.*, vol. 157, pp. 499-514, 2009.

[18] V. D. Putte and P. Part, "Oxygen and chromium transfer in prefused gills of rainbow trout, salmo gairdneri exposed to hexavalent chromium at two different pH levels," *Aquat. Toxicol.*, vol. 2, pp. 31-45, 1982.

[19] A. M. Yousafzai, M. Siraj, H. Ahmad, and D. P. Chivers, "Bioaccumulation of heavy metals in common carp: implications for human health," *Pakistan. J.*, vol. 44, pp. 489-494, 2012.

[20] R. Vinodhini and M. Narayanan, "Bioaccumulation of heavy metals in organs of fresh water fish cyprinus carpio (Common Carp)," *Int. J. Environ. Sci. Tech.*, vol. 5, pp. 179-182, 2008.

[21] J. Bressler and G. Goldstein, "Mechanisms of lead neurotoxicity," *Biochem. Pharmacol.*, vol. 41, pp. 479-84, 1991.

[22] D. Holtzman, C. Devries, H. Nguyen, and K. Bensch, "Maturation of resistance to lead encephalopathy: Cellular and subcellular mechanisms," *Neurotoxicology*, vol. 5, p. 124, 1984.

[23] O. Mestek, Z. Deyl, I. Miksik, J. Novotna, I. Pfeifer, and J. Herget, "Accumulation of lead in tissues after its administration in drinking water to laboratory rats," *Physiol. Res.*, vol. 47, pp. 197-202, 1998.

[24] J. G. Singh, I. C. Yen, V. A. Stoote, and L. Chatergoon, "Distribution of selected heavy metals in skin and muscle of five tropical marine fishes," *Environmental Pollution*, vol. 69, pp. 203-215, 1991.

[25] N. Naghshbandi, S. Zare, R. Heidari, and S. S. Polcheglu, "Bioaccumulation of lead nitrate in freshwater crayfish (Astacus Leptodactylus) tissues under aquaculture conditions," *Pakistan Journal of Biological Sciences*, vol. 10, pp. 3245-3247, 2007.

[26] S. Smirjakova, O. Ondrasovicova, A. Kaskova, and K. Lakticova, "The effect of cadmium and lead pollution on human and animal health," *Folior Veterinaria*, vol. 49, pp. S31-S32, 2005.

[27] K. Tsuzuki, M. Sugiyama, and N. Haramaki, "DNA signle strand break and cytotoxicity induced by chromate (VI), cadmium (II) and mercury (II) in hydrogen peroxide resistant cell lines," *Environ. Health Perspect*, vol. 102, pp. 341-342, 1994.

[28] K. S. Kasprzak, "Oxidative DNA and protein damage in metal induced toxicity and carcinogenesis," *Free Radical Biol Med.*, vol. 32, pp. 958-67, 2002.

[29] N. Ercal, H. Gurer-orhan, and N. Aykin-Burns, "Toxic metals and oxidative stress part I: Mechanisms involved in metal-induced oxidative damage," *Current Topics in Medicinal Chemistry*, vol. 1, pp. 529-539, 2001.

[30] J. J. Scott-Fordsmand, "Toxicity of nickel to soil organisms in denmark," *Rev. Environ. Contam. Toxicol.*, vol. 148, p. 1, 1997.

[31] M. Mela, M. A. F. Randi, D. F. Ventura, C. E. V. Carvalho, E. Pellerier, and C. A. O. Ribeiro, "Effects of dietary methyl mercury on liver and kidney histology in the neotrophical fish Hoplias malabaricus, ecotox," *Environ. Saf.*, vol. 68, pp. 426-435, 2007.

[32] T. Kimura and K. Doi, "Effects of all-trans-retinoic acid on the dorsal skin of hairless dogs," *Toxicologic Pathology*, vol. 26, pp. 595-601, 1998.

[33] R. O. Gallucci, S. Dell, D. Rabe, and L. Fechter, "JP-8 jet fuel exposure induces inflammatory cytokines in rat skin," *International Immuno Pharmacology*, vol. 4, pp. 1159-1169, 2004.

[34] A. H. Batawy, "Effect of gasoline contact on histological and histochemical structures of guinea pig skin," Ph.D Thesis, King Abdulaziz University, 2008.

[35] F. Muhammed, N. Monteiro-Riviere, and J. Riviere, "Comparative in vivo toxicity of topical JP-8 Jet fuel and its individual hydrocarbon components: Identification of tridecane and tetradecane as key constituents responsible for dermal irritation," *Toxicologic Pathology*, vol. 33, pp. 258-266, 2005.

[36] J. R. King and Monteiro-Riviere, "Cutaneous toxicity of 2-chloroethyl methyl sulfide in isolated perfused porcine skin " *Toxicology and Applied Pharmacology*, vol. 104, pp. 167-179, 1990.

[37] N. A. Monteiro-Riviere, A. O. Imman, and J. E. Riviere, "Skin toxicity of jet fuels: Ultrastrucural studies and the effects of substance P.," *Toxicology and Applied Pharmacology*, vol. 195, pp. 339-347, 2004.

[38] S. Tość-Luty, D. Przebirowska, J. Latuszyńska, and M. Tokarska-Rodak, "Histological and ultrastructural studies of rats exposed to carbaryl," *Ann Agric Environ.*, vol. 8, pp. 137-144, 2001.

Table-1. Concentrations of benzene and some heavy metals in both types of gasoline (90 and 80).

Type of gasoline	**Gasoline contents**			
	Benzene %(v/v)	Lead mg/l	Cadmium mg/l	Nickel mg/l
Gasoline 90	0.897	0.241	0.013	0.009
Gasoline 80	1.17	0.399	0.098	0.023

Table-2. Bioaccumulation of some heavy metals (%total) in skin male mice topically exposed to both types of gasoline (90 and 80)

Experimental group	**Lead**	**Cadmium**	**Nickel**
Control	$0.13^{d} \pm 0.13$	$0.20^{c} \pm 0.06$	$0.10^{d} \pm 0.06$
gasoline 90 /4 weeks	$3.87^{c} \pm 0.30$	$1.40^{bc} \pm 0.26$	$0.43^{cd} \pm 0.07$
gasoline 90 / 8 weeks	$4.57^{bc} \pm 0.45$	$2.77^{ab} \pm 0.73$	$0.87^{bc} \pm 0.09$
gasoline 80 / 4 weeks	$6.0^{b} \pm 0.97$	$3.33^{a} \pm 0.34$	$1.50^{ab} \pm 0.25$
gasoline 80 /8 weeks	$10.07^{a} \pm 0.71$	$3.80^{a} \pm 0.57$	$1.67^{a} \pm 0.44$
F (p)	37.05^{*} (<0.001)	10.54^{*} (0.001)	8.26^{*} (0.003)

F: F test f (ANOVA)
p: p value for F test (ANOVA)
Different superscripts are significant.
*: Statistically significant at $p \leq 0.05$

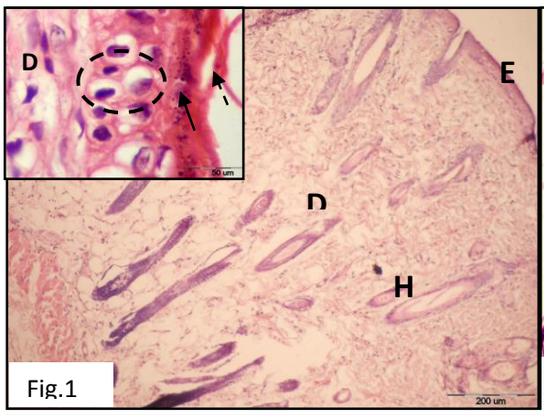
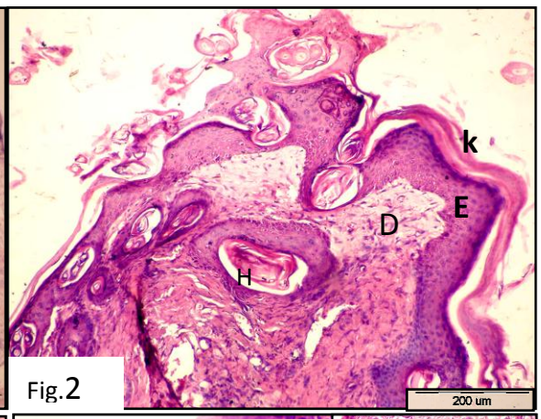


Fig.1



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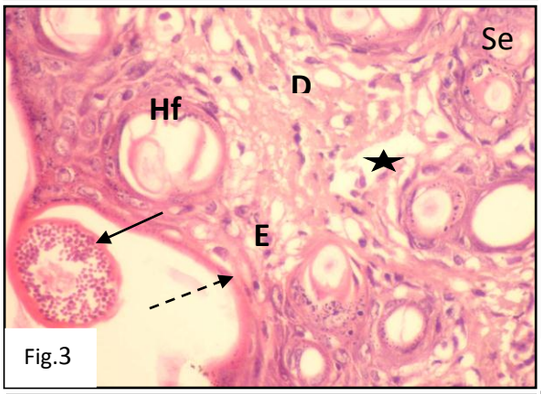

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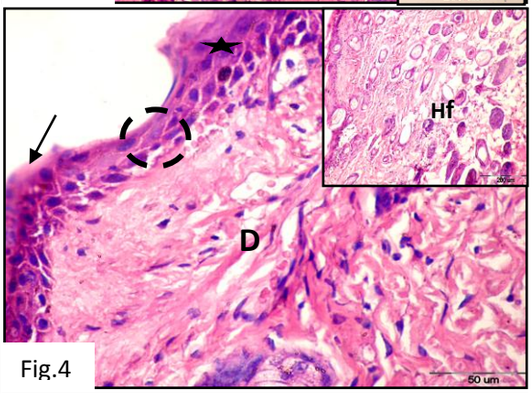

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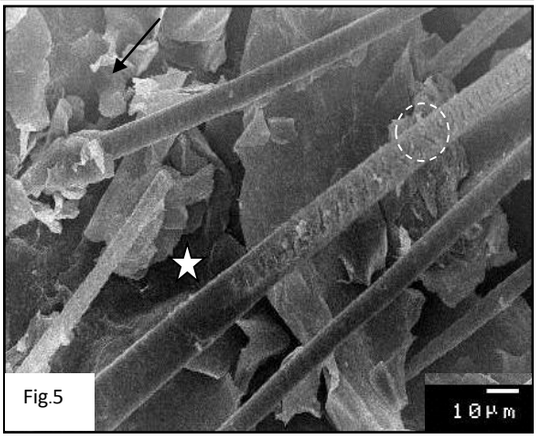

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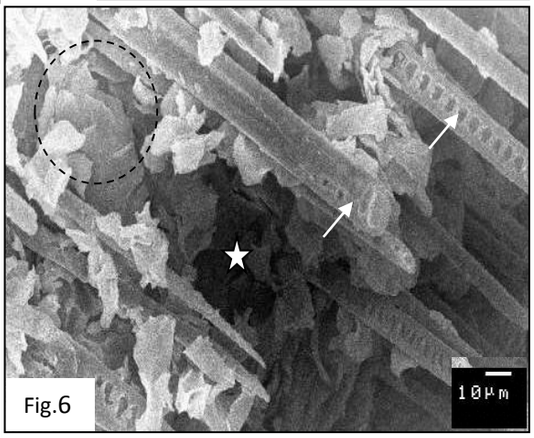

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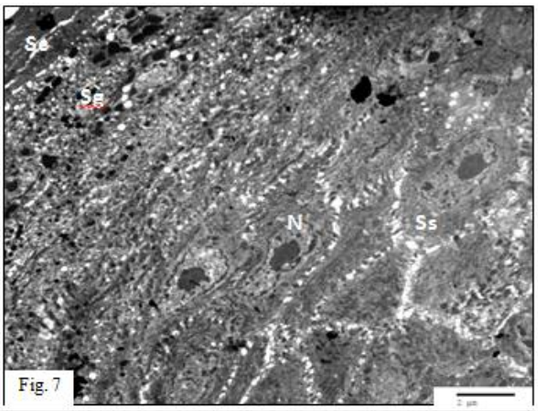

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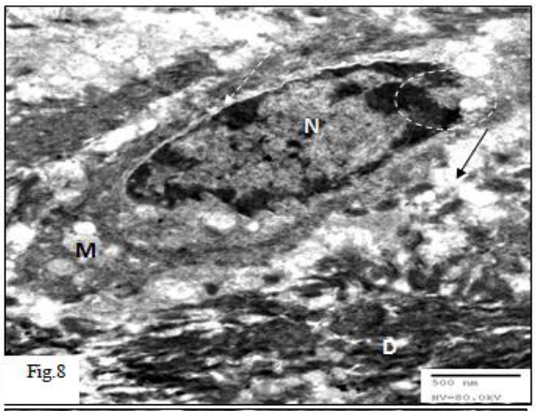

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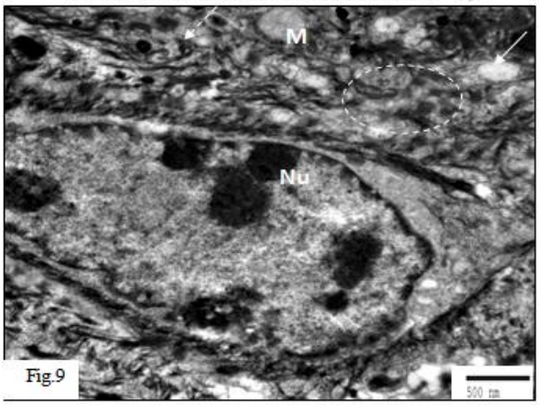

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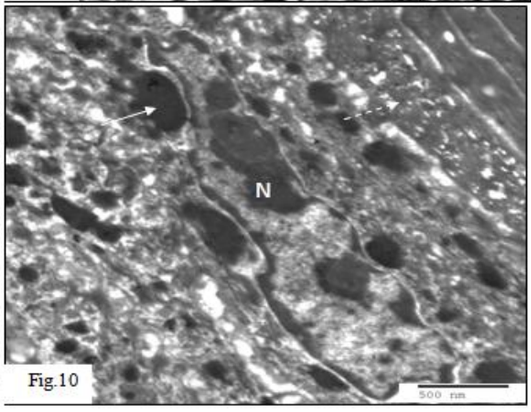

Fig.10

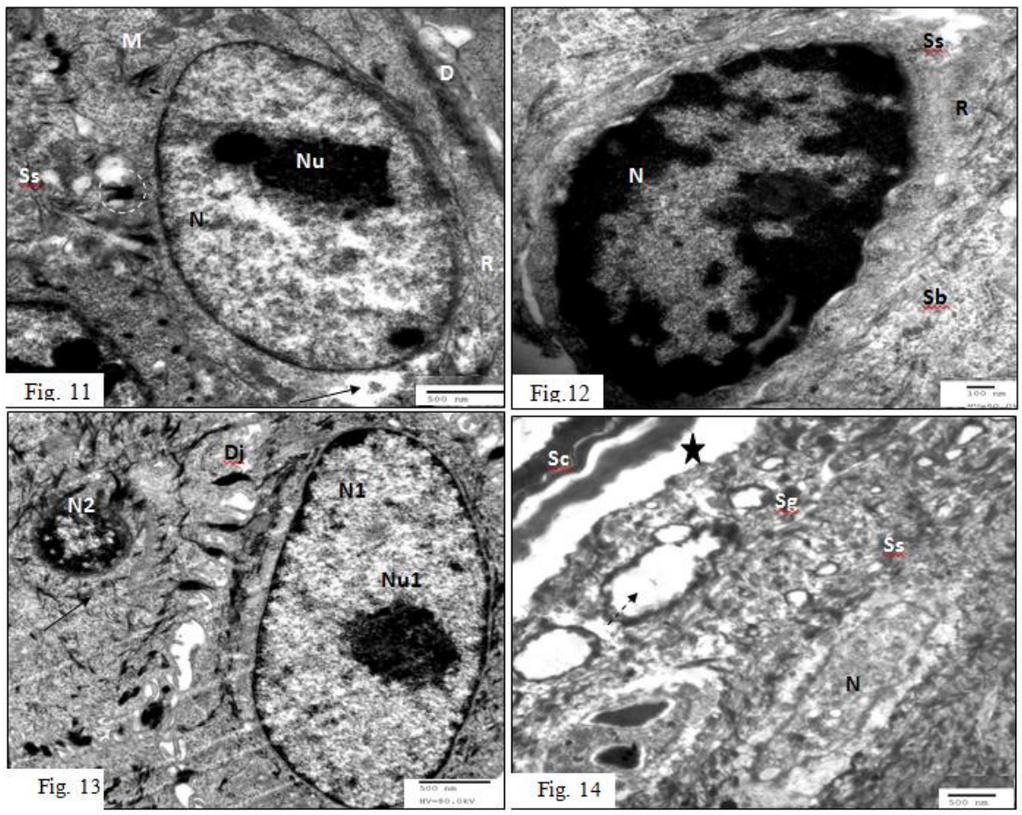

Fig-1. Light micrograph of vertical section of male mouse thin skin topically exposed to gasoline 90 for 4 weeks, showing increased number of hair follicles (H) in dermis (D). Epidermis (Ep) (H&E, X10).Inserted part showing: detached keratin (dashed arrow), thick dark stratum granulosum (arrow), stratum basal depecting paranuclear vacuolization and pyknotic nuclei (circle). Degenerated dermis (D) (H&E, X100).

Fig-2. Light micrograph of vertical section of male mouse thin skin topically exposed to gasoline 90 for 8 weeks, showing abnormal skin architecture with detached thick keratin (K), hyperplasia of epidermis (Ep) and increased hair follicles number (H) in destructed dermis (D) (H&E, X10).

Fig-3. Light micrograph of vertical section of male mouse thin skin topically exposed to gasoline 80 for 4 weeks, showing thin stratum corneum (dashed arrow), subcorneal microabscesse (arrow) filled with red blood cells in epidermis (Ep) with hyperplasia and lytic dermis (*) , increased hair follicle number (H) and sebaceous gland (SE). Dermis (D) (H&E, X40).

Fig-4. Light micrograph of vertical section of male mouse thin skin topically exposed to gasoline 80 for 8 weeks, showing absence of stratum corneum (arrow), epidermis with alternated thin (circle) and thick areas (star) and obvious increase in hair follicles number (H) in the dermis (D) (H&E, X10).

Fig-5. Scanning electron micrograph of skin of mouse topically exposed to gasoline 90 for 8 weeks, showing destructed thick hair shaft (circle) with broken hair-sheath and increased hair number. Note, stratum corneum units separation (star) with detached scales from the skin surface (arrow) taken spicules and polygonal shapes (Gold coated sample, X750).

Fig-6. Scanning electron micrograph of skin of mouse topically exposed to gasoline 80 for 8 weeks, showing many thick destructed hair shafts (arrows) with broken hair sheath and stratum corneum units separation (star) with detached variable shapes spicules (Gold coated sample, X750).

Fig-7-10. Transmission electron micrograph of vertical section of mouse thin skin topically treated with gasoline 90 for 8 weeks :

Fig-7. showing ,hyperpalsia of both stratums spinosum (Ss) with flate nuclei (N) in wide separated cells and stratum granulosum (Sg). Stratum corneum (Sc) . (uranyl acetate-lead citrate stain, x1000).

Fig-8. showing, basal keratinocyte having irregular nucleus (N) with densely packed heterochromatin, dilated nuclear envelope (dashed arrow) and cytoplasmic invagination inside the nucleus (circle). Dark cytoplasm with accumulation of tonofilaments at perinuclear area, many cytoplasmic vacuoles (arrow) and degenerated mitochondria (M). dermis (D) (uranyl acitate-lead citrate stain, x4000).

Fig-9. showing ,part of spinosum keratinocyte with vesicles (arrows) , accumulated tonofilament (dashed circle), round keratinohyaline granules (dashed arrow) and irregular nucleus (N) (uranyl acetate-lead citrate stain, x4000).

Fig-10. showing ,granulosum keratinocyte with increased keratinohyaline patches (arrow), flate elongated nucleus (N) having dilated nuclear envelope and chromatin masses in dense cytoplasm. Vacuolated stratum corneum (Sc) (uranyl acetate-lead citrate stain, x4000).

Fig-11-14. Transmission electron micrograph of vertical section of mouse thin skin topically treated with gasoline 80 for 8 weeks :

Fig-11. showing **,**basal keratinocyte with pale lytic nucleus (N) and nucleolus (Nu), cytoplasmic vacuole (arrow), decreased amount of tonofilaments (dashed arrow), desmosomal junction (circle), degenerated mitochondria (M) and free ribosomes (R). Dermis (D)and stratum spinosum (Ss) (uranyl acetate-lead citrate stain, X4000)

Fig-12. :showing necrotic spinosum keratinocyte with nucleus undergoing pyknotic state in cytoplasm losting its tonofilaments and with few free ribosomes (R). Stratum basal (Sb), Stratum spinosum (Ss) (uranyl acetate-lead citrate stain, X7500).

Fig-13. showing , stratum spinosum with keratinocyte has lytic pale nucleus (N1) with clear nucleolus (Nu1), peripheral keratin tonofilaments (dashed arrow). Necrotic spinosum keratinocyte with pyknotic nucleus (N2) has distributed cytoplasmic keratin tonofilaments (arrow). Note desmosomal junction (Dj) (uranyl acetate-lead citrate stain, X4000).

Fig-14. showing, spinosum keratinocyte with karyolytic nucleus (N) in degenerated cytoplasm, degenerated keratinohyaline patches (dashed arrow) in stratum granulosum (Sg) and destructed stratum corneum (Sc). Note, the expansion of intercellular space (star). Stratum spinosum (Ss) (uranyl acetate-lead citrate stain, X2000).