



Original Research Article

Histopathologic effect of Xylene and Ultraviolet Type B exposure on mouse skin

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ABSTRACT

Keywords

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Statistical
analysis

The aim of the present study was to investigate the role of UVB in synergizing toxicity effect of xylene on mouse skin. Forty adult albino *Mus musculus species*, *BALB/c strain* mice were used and designed into four groups, Group A (Control group, n=10) which was not exposed to UVB and Xylene, Group B (n=10) exposed to Xylene, Group C (n=10) which were exposed to UVB light only, while the remainder group (Group D, n=10) were exposed to Xylene and UVB. Mice from group B and D were exposed to 1ml of xylene with mice from group C exposed to UVB for 30 minutes 4days/week (6 weeks). The control group showed normal epidermal thickness, the mean of the group was equaled as 7.035 μ m, in xylene group there was moderately increased of epidermal thickness as recorded as 37.064 μ m, In group C there was a huge epidermal thickness and recorded as 136.341 μ m, while epidermal thickness was slightly decreased in Xylene and UVB group and registered as 73.971 μ m, in comparison to UVB exposed group. We demonstrated that UVB increased the effect of xylene several fold in relation to effect of xylene alone that have not been recorded in Xlyene group.

Introduction

The skin cancer incidence has increased substantially over the past decades and the role of ultraviolet radiation in the etiology of skin cancer is well established (Armstrong and Kricger, 2001), most ultraviolet (UV) light on earth comes from the sun, and it is classified by International Commission on Illumination into three groups: Ultraviolet type A (UVA, long wave) 320-400nms, ultraviolet type B (UVB, medium wave)

290-320nm and ultraviolet type C (UVC, short wave) 200-290nms (Pentland et al., 1999).

UVB is commonly considered as the most harmful part of the UV-spectrum due to its DNA damaging potential and well known carcinogenic effect. Acutely, exposure to UV induces cellular damage and initiates repair responses in epidermal keratinocytes.

Chronic UV irradiation causes repeated epidermal cell damage and neoplasia (Bruce and Brodland, 2000). The skin reacts to UV injury through activation of numerous signaling pathways that alter transcription factors and it is well described these responses consists of erythema, increased keratinocyte proliferation and differentiation, modified inflammation, and increased cytokine production (Tyrrell, 1996; El-Abaseri et al., 2006; Thomas-Ahner et al., 2007).

Xylene is an aromatic hydrocarbon widely used in the manufacture of insecticides, pharmaceuticals, as a component of detergents, also as a solvent for paints, inks, and adhesives. Xylene-containing petroleum distillates are used broadly and increasingly in blending petrol (USEPA, 1988; Cancer, 1990) It is found in small amounts in airplane fuel, gasoline and cigarette smoke. In histological laboratories xylene is used for tissue processing, staining and cover slips. Its high solvency factor permits extreme dislocation of alcohol and renders the tissue apparent, improving paraffin infiltration. In staining procedures, it has intense de-waxing and clearing competences that contribute to brilliantly stained slides (Edwards and Campbell, 1984; ATSDR, 1995).

Further industrial exposure, the foremost pathway of human interaction or contact is through soil contamination from seeping underground storage tanks containing petroleum products. Xylene can leak into the soil, surface water or ground water where it may persist for months or more before its' decomposed into other chemicals. However, as it evaporates easily, most of it goes into the air, then formed a less harmful chemical by action of sunlight (Kandyala et al., 2010).

Xylene isomers are readily absorbed after inhalation, withholding percentages of 60–

65% in humans. They are absorbed to some extent (exact percentages not known) via the skin; the few data available indicate rapid distribution of the compound after exposing. Xylenes can cross the placenta. They are deposited in adipose tissue in both laboratory animals and humans (ECETOC, 1986; Janssen et al., 1989). Carcinogenicity studies in rats and mice provided some relevant information on the toxic effects of xylenes after oral administration (NTP, 1986). The aim of the present study was to investigate the role of UVB in synergizing toxicity effect of xylene on mouse skin.

Materials and Methods

Animal model

Forty adult albino *Mus musculus species, BALB/c strain* mice (20 males and 20 females) were used in this experiment, each of them weighing 20-30 mg which was fed with standard pellet diet (Pico Lab) and provided with water ad libitum. Animals were housed in the animal house Department of Biology/ School of Science/Sulaimani University under a controlled room temperature of about 25 °C and photo-periodicity of 12 hours light/dark system.

Animals were assigned into four groups; Group A (Control group, n=10) which were not exposed to UVB and Xylene spraying, Group B (n=10) exposed to Xylene through spraying the mouses' back skin, Group C (n=10) which were exposed to UVB light only, while the remainder group (Group D, n=10) were exposed to Xylene and UVB.

Xylene exposure

Xylene that was used in this experiment (SURECHEM product LTD, NEEDHAM MARKET SUFFOLK ENGLAND). Mice

from both groups (Group B and D), the shaved area was exposed to 1ml of xylene. This process was performed 4days/week for 6 consecutive weeks.

UVB Lamp

The Lamp which was used in this experiment was 312 nm wavelength, 15 Watts, VILBER-LOURMAT-FRANCE, with a calculated power 80mj/Sec. Mice from all groups with the exception of group A and B were exposed to UVB light for 30 minutes 4 days/week (6 weeks) consecutively, and this was done after shaving the mouse's back skin (2*5 cm).

Sampling method

At the end of the experiments, the animals were euthanized using (Xylazine-Ketamine: 0.1ml/10g of body weight) as recommended dose intraperitoneally (In a sterile 10 ml tube with a rubber stopper, mix 1ml of ketamine (100mg/ml) + 0.1ml of xylazine (100mg/ml) + 8.9ml of sterile water for injection). Tissue samples were taken from dorsal back skin. The tissue specimens were fixed at 10% neutral buffered formalin for at least 24 hours and then routinely processed that will be performed in the Pathology research Lab/School of Medicine, Sulaimani University. The samples were embedded paraffin and section at a 5µm thickness to detect any abnormal lesions which might be formed by UVB and Xylene exposure.

Histomorphometry

Sections of the dorsal skin were checked out under ordinary light microscope; the measurement and calculation of epidermal thickness from each histological sections were carried out using an image analyzer (Scope image software 9.0 "H3D" computer system-England, digital binocular compound

microscope) in the Histology Department/College of Science/Salahaddin University/Erbil. The full epidermal thickness was examined by the measuring of epidermal length from the top layer (stratum corneum) to the bottom layer (stratum basale) of different five Fields (X100 power magnification), which representing the epidermal thickness, and then the mean was calculated for each sample.

Statistical analysis

For each cases epidermal thickness measured and the mean was obtained, then the mean of the whole control group used as standard for comparing the epidermal skin thickness in other cases of different groups. The Student's t-test (paired) and Pearson's correlation coefficients were used to calculate the skin thickness of the back region and in all analyses, a *P* value of <0.05 was considered to be significant statistically.

Result and Discussion

Histopathological changes

Grossly control group showed normal skin appearance without evidence of erythema, congestion or thickness formation by palpation detected as in (Fig.1a), multiple gross lesion was observed in group B such as redness of skin back region due to vasodilation, alopecia in some region with sloughed dry scaling skin as in (Fig.1b), the results obtained in the present study agree with many studies (Engström et al., 1977; Riihimäki, 1979; Saito et al., 2011), whom detected that human skin exposure to xylene causes skin irritation, dryness, scaling of the skin, and vasodilation. In studies of Anderson et al.; Consumer Product Testing; Food and Drug Research Labs, who proved that dermal effects of xylenes in laboratory

animal included; rabbits, guinea pigs, and mice induced Mild-to-severe skin irritation that ranged from erythema and edema to epidermal thickness (Anderson et al., 1986; Consumer Products Testing, 1976; FAD Research Laboratories Incorporated, 1976a.). These effects of xylene may be due to that Xylenes are absorbed dermally to a much lesser extent than by inhalation or oral exposure, especially following dermal exposure to xylene vapor which is consistent with Riihimaki and Pfaffli 1978. While in group C showed variable sized-nodular thickness or light brown coloration as in (Figure 1c), there was a variable sized nodular thickness of red-yellow color in group D as in (Fig.1d).

We demonstrated the variable degree of epidermal thickness in response to chronic exposure to UVB and Xylene spray with H&E stains. The control group shows normal epidermal thickness as in (Fig.2a), where the mean of the group was calculated as $7.035\mu\text{m}$, in xylene group there was slightly increased of epidermis as recorded as $37.064\mu\text{m}$ as in (Fig.2b), There was a huge epidermal thickness due to development of acanthotic seborrheic keratosis in UVB group and recorded as $136.341\mu\text{m}$ (Fig.2c), which is indicated that UVB with a long duration initiated and promoted the seborrheic keratosis development, our results were consistent with the previously published papers of (Haw et al., 2009; Snur, 2011; Saeed and Salmo, 2012), who showed a relation between UVB and SK development. While epidermal thickness was slightly decreased in Xylene and UVB group and registered as $73.971\mu\text{m}$ as in (Fig.3d), in comparison to UVB exposed group. We detected a rather significant effect between UVB and xylene group this is due to the combined effect of xylene and UVB exposure on the skin, our work provided evidence that UVB synergize the effect of xylene because Xylene only

cannot produce such effect and controversially xylene reduced the UVB effect as mentioned by previous studies proved that Xylene induced Mild-to-severe skin irritation that ranged from erythema and edema to epidermal thickness (Anderson et al., 1986; Consumer Products Testing, 1976; FAD Research Laboratories Incorporated, 1976a.). Dermal initiation/promotion of two previous studies (Pound, 1970; Pound and Withers, 1963), who suggesting that xylene may be a promoter of skin cancer and might also act as an initiator or co-carcinogen. Under the Draft Revised Guidelines for Carcinogen Risk Assessment, human and animal data are inadequate for an assessment of the carcinogenic potential of xylenes (U.S. EPA, 2002). Previous studies proved that when workers occupationally exposed to solvents and examined for the cancer and leukemia risks, they suggest a possible relationship between coal-based xylene exposure and leukemia (Arp Jr et al., 1983; WILCOSKY et al., 1984).

(Mean±S. E. M) of different group by using T test and Pearson coefficient correlation test

The measurement of epidermal thickness for the different cases of the control one is presented in (Table 1 and 2) and Fig.3. The mean of control group (Mean±SEM = 7.035 ± 0.086), the full epidermal thickness of Xylene exposure cases were moderately increased than the control group which was (37.064 ± 3.171), while epidermal thickness severely increased in UVB exposure, which was (136.341 ± 8.776), whereas UVB and Xylene group value was (73.971 ± 6.61817), which was statistically highly significant with $P=0.000$ according to T test and there was a strong correlation of skin thickness between individual group with $r_{\text{Pearson correlation}}=1$, $P=0.000$ according to Pearson's correlation coefficient test.

Table.1 Measurement of epidermal thickness (Mean±SEM) value of individual and groups

	Control	Xylene	UVB	Xylene and UVB
MEAN±SEM	7.035±0.086	37.064 ±3. 171	136.341±8.776	73.971±6.61817
Probability				
Control		0.00	0.00	0.00
Xylene	0.00		0.00	0.00
UVB	0.00	0.00		0.001

*P<0.05 Student 't' test; paired observations

Table.2 Measurement of epidermal thickness of individual and different group by using Pearson's correlation coefficient test

	Control	Xylene	UVB	Xylene and UVB
Control	1			
Xylene	0.954	1		
UVB	-0.916	-0.945	1	
Xylene and UVB	0.921	0.984	-0.933	1

** Correlation is significant at the 0.01 level (2-tailed)

Figure.1 A: Normal skin appearance in the control group, B: Scaling skin, redness with sloughing of hair only small part remained in Xylene group, C: A velvety to nodular, light brown skin in a mouse of the exposed group (arrow), D: variable sized nodule of yellow-red color in Xylene and UVB exposed group

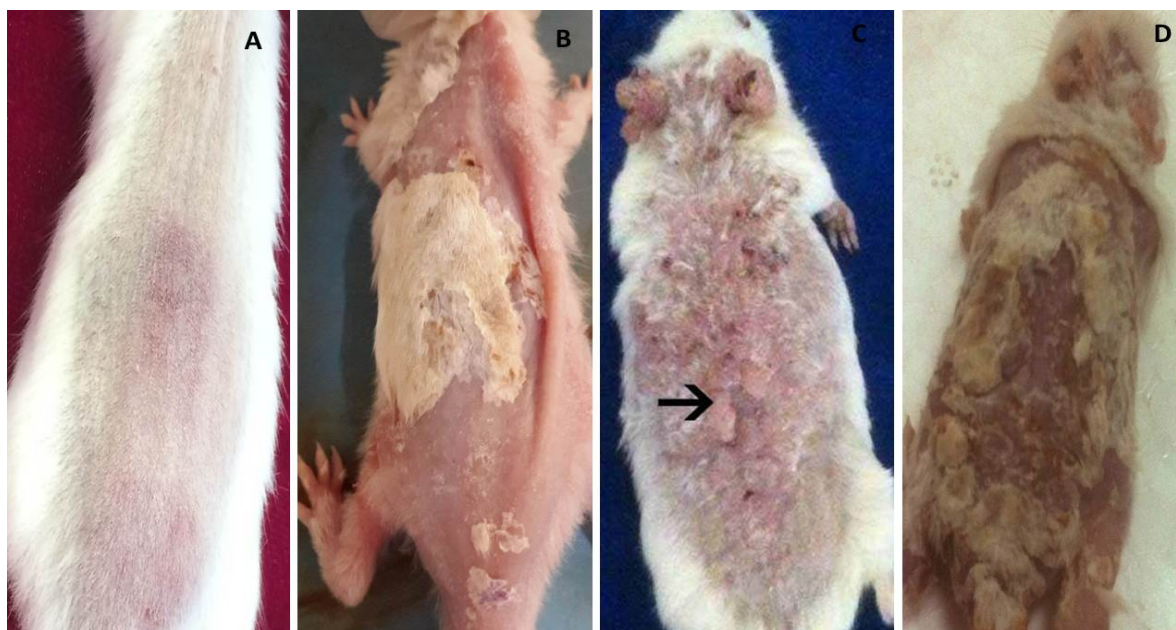
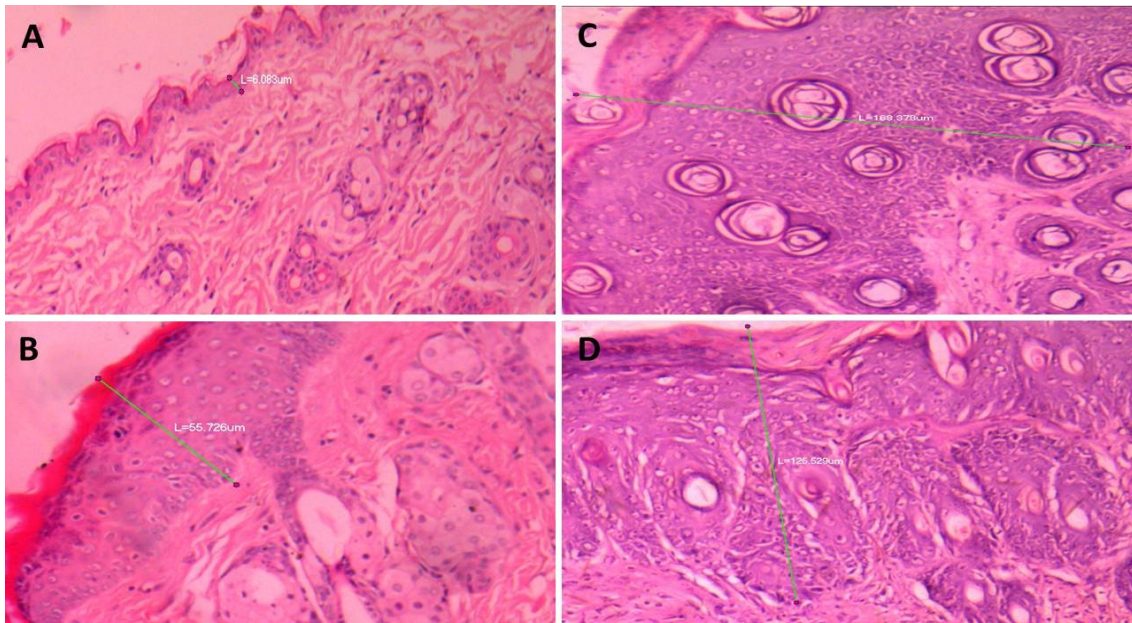
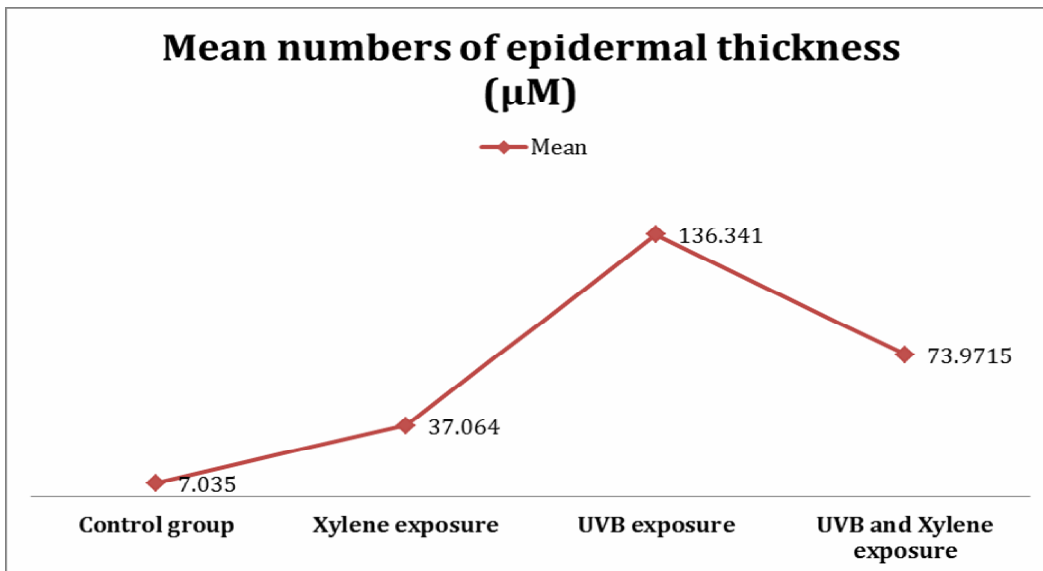


Figure.2 Calibrated epidermal proliferation in different group. A: Control group (H&E stains, 100), B: Xylene group (H&E stains, zoom in), C: UVB group (H&E stains, zoom in), D: UVB and Xylene group (H&E stains, zoom in)



Figur.3 Line chart shows mean numbers in different groups



A comparison of B to D and C group result was significant, according to T test with P= 0.000, also there was a highly significant difference between C exposure group to D

exposure group with P= 0.001 according to T test.

This study showed a crucial correlation of mean epidermal skin thickness between

different groups (Table 2) with opposite direction; A negative correlation was found between the mean skin thickness of xylene and UVB group ($r_{\text{Pearson correlation}}=-0.945$, $P=0.000$), a positive, strong correlation was observed between mean skin thickness of B with D exposure group ($r_{\text{Pearson correlation}}=0.984$, $P=0.000$), whereas a strong negative correlation was noted between UVB with Xylene and UVB exposure group ($r_{\text{Pearson correlation}}=-0.933$, $P=0.000$). Up to our knowledge, no study has ever been published on this correlation and so this study was the first to prove this.

We demonstrated that UVB increased the effect of xylene several fold in relation to effect of xylene alone that have not been recorded in Xylene group.

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