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The Effect of Subchronic Exposure of Petrol Vapours on Leukocyte Parameters in Rats

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Abstract. Petrol is one of natural products of petroleum which contains different types of hydrocarbons including volatile organic compounds. The exposure of these hydrocarbons can affect human health. Thus, this study was done to investigate the effect of subchronic exposure of petrol vapours on leukocyte parameters in rats. Fourteen healthy male Sprague Dawley rats weighing about 190±10g were divided into control and exposed group. Petrol was exposed through whole-body inhalation chamber for 8 hours per day, 5 days per week for 30 days. The blood samples were then collected and tested for leukocyte parameters including total leukocyte count (TLC), differential leukocyte count (DLC) and neutrophil phagocytic activity (NPA). There were significant reduction of TLC, neutrophil percentage and NPA in exposed group. In conclusion, the result suggested that 30 days exposure of petrol vapours did induce leukocytopenia and disruption of phagocytic activity in rats.

Keywords: Petrol, Leukocyte parameters, Leukocytopenia.

1. Introduction

Petrol is one of natural products of petroleum, and parts of fractional distillation of crude petroleum instead of kerosene and diesel [1]. Petrol is highly flammable, easily evaporate and can form explosive mixture in the air. Petrol is widely used in some electricity generating machinery and as fuel for automotive vehicles. Daily applications of petrol have resulted in direct exposure to general population however; the most affected are those who occupationally exposed to the fumes [2]. Primary constituents of petrol, petroleum hydrocarbons may directly affect human health. These hydrocarbons include aromatic hydrocarbon and polynuclear aromatic hydrocarbons (PAHs). Aromatic hydrocarbon is recognized as notorious chemicals for health risk assessment at most petroleum contaminated sites. These aromatic hydrocarbons include BTEX (benzene, toluene, ethylbenzene and xylene) compounds [3]. These compounds are the simplest alkylbenzenes and the major sources of BTEX compounds are from evaporative losses from petrol as well the internal combustion process itself. Even though these chemicals only make up a small portion of petrol, they are among of the most potentially dangerous once released into environment [3]. Previous report stated that prolonged occupational exposure of petrol vapours produced deleterious effects such as genotoxic, mutagenic, immunotoxic, carcinogenic and neurotoxic manifestations on various organs and systems, which include respiratory, immune and nervous systems [1].

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Thus, the key objective of this study was to investigate the effect of subchronic exposure of petrol vapour on some leukocyte parameters in rats namely, total leukocyte count (TLC), differential leukocyte count (DLC) and neutrophil phagocytic activity (NPA).

2. Materials and Methods

2.1. Test Animals

Total of 14 healthy male Sprague Dawley rats weighing about 190±10g were used in this experiment. They were housed in standard cages [4] and they had been left acclimatized for 7 days to laboratory conditions and handling before the commencement of the experiment [5]. During the acclimatization period, the animals were fed with pellet rat chow and water ad libitum [4].

2.2. Petrol Samples

Petrol used for this study is commercially known as Ron95 and was purchased from Petronas petrol filling station near the Universiti Kuala Lumpur, Institute of Medical Science Technology Kajang, Selangor, Malaysia. Two hundred (200) ml of petrol was daily used for 30 days.

2.3. Exposure to Petrol Vapours

Rats were exposed to petrol vapour by inhalation for 7 hours per day, 5 days per week for 30 days. The exposed group was placed in the modified whole-body inhalation chamber measuring 150cm x 90cm x 210cm. The modified chamber had two air inlets (60mm in diameter) which one of them attached with cooling fan for ventilation and wire as a wall to prevent the rats get closed to the beaker containing petrol. Two 500 ml beakers containing 200 ml of petrol were placed in the chamber in order to optimize the petrol evaporation in the chamber area and the animals were allowed to inhale the vapour evaporating from the beakers and they were deprived from food or drinks. After the exposure duration, the animals were removed from the inhalation chamber and were returned to the standard cages. The petrol amount were monitored and kept at 200 ml during whole exposure period to ensure the constant presence of petrol volatile material [5], [6].

2.4. Blood Samples Collection

At the end of the experimental periods, blood samples from both groups were collected for the assessments. The rats were anesthetized by whole body exposure of diethyl ether. A cotton ball soaked in the exact amount of diethyl ether was placed in a glass desiccator, under a screen to avoid direct skin contact with the soaked cotton. Each rat was monitored after being placed inside the desiccator with a tightly closed lid. The decline of respiratory rate (approximately 50 %) and loss of the righting reflex were considered to be signs of deep anesthesia which followed approximately 4 minutes post-exposure of diethyl ether. The rat was immediately removed from the desiccator as soon as these signs were observed and a cardiac puncture was performed immediately. The blood samples were transferred into EDTA blood tube, plain tube and heparinized plain tube. The EDTA tube was used to calculate TLC and examined DLC. Meanwhile, plain tube was used to obtain rat serum and heparinized plain tube was used to determine phagocytic activity of neutrophils. At the end of the experiments, the rats were euthanized by carbon dioxide [7].

2.5. Leukocyte Parameters

2.5.1. Total Leukocytes Count (TLC)

TLC is a test to measure the number of leukocytes in the body and was done in this study to investigate whether petrol exposure has effect on leukocyte numbers in the body circulation. Turk's solution was used as leukocyte diluting fluid and contained weak acid (Glacial acetic acid 2%v/v) to lyse the erythrocytes and to stain the nucleus of the leukocytes [8].

2.5.2. Differential Leukocyte Count (DLC)

DLC is a test to measure the percentage of each leukocyte types and was done in this study to investigate whether petrol exposure has effect on certain types of leukocytes [8].

2.5.3. Neutrophil Phagocytic Activity (NPA)

This method was done to assess neutrophil's intracellular killing ability. Peripheral blood neutrophils were first isolated by using commercially available separation media Histopaque. Methylene blue was used as the indicator dye and the test organism was *Candida albicans*. Phagocytosis was measured by counting, microscopically, the number of ingested yeast within the neutrophils per hundred of counted neutrophils. This technique provides a rapid, inexpensive and easily quantified assay for assessing discrete phagocytic cell functions [9].

2.6. Statistical Analysis

Data analysis was performed using the statistical Package for the Social Sciences software (SPSS). The value of leukocyte parameters was reported as mean ± standard error of mean (SEM). The differences between control and exposed groups were tested by using student t-test. The differences among groups were considered significant when $p < 0.05$ [8].

3. Result

There was significant decrease in TLC ($x10^3/mm^3$) of exposed group ranging between 17.86 ± 0.56 and 14.24 ± 0.45 (Table 1) as compared to control group. There was also significant differences of DLC (%) between exposed and control groups in this experiment (Table 2). The leukocytes were identified by their nuclear lobes and cytoplasmic granules. The neutrophil percentage in exposed group was lower (35.00 ± 1.13) than control group (22.57 ± 0.81). The same reduction also seen in the eosinophil percentage of exposed group (2.43 ± 0.202). On the contrary, the basophil, lymphocyte and monocyte percentages were higher in control group as compared to rats that exposed to petrol vapour for 30 days. In addition, Table 3 shows the neutrophil phagocytic activity of both groups. The result was recorded according to the percentage of engulfment (%) of the neutrophils and the percentage of neutrophil engulfment in exposed group was significantly lower (38.86 ± 1.22) than control group (86.14 ± 0.74)

Table 1: The effects of petrol vapour on total leukocyte count ($x10^3/mm^3$) in control and exposed rats

Parameter	Control	Expose
Total leukocyte count	17.857 ± 0.562*	14.236 ± 0.451*

*: significant $p < 0.05$

Table 2: The effects of petrol vapour on differential leukocyte count (%) in control and exposed rats.

Parameter	Control	Expose
Neutrophil count	35.00 ± 1.134*	22.57 ± 0.812*
Eosinophil count	3.43 ± 0.202*	2.43 ± 0.202*
Basophil count	0.43 ± 0.202*	1.71 ± 0.184*
Lymphocyte count	56.71 ± 0.993*	61.43 ± 0.782*
Monocyte count	4.43 ± 0.202*	11.86 ± 0.595*

*: significant $p < 0.05$

Table 3: The effects of petrol vapour on the neutrophil phagocytic activity (%) in control and exposed rats.

Parameter	Control	Expose
Percentage of engulfment	86.14 ± 0.738 *	38.86 ± 1.223*

*: significant $p < 0.05$

4. Discussion

We are living in the environment that comprises of at least 100,000 prevalent chemicals and to which approximately 100 new compounds are added each year [10]. Among these compounds, crude oil and petrol are potentially toxic to living things [11]. Thus, this study shows that petrol exposure has caused significant alteration in leukocyte parameters. Leukocytes act as scavengers in order to fight infections, defend the body by phagocytosis against invasion by foreign organism and to produce and distribute antibodies in immune response. In this study, Table 1 shows distinct reduction of TLC of exposed rats as compared to control. Similar results were recorded in previous studies which significant reduction of TLC in animals that exposed with benzene and crude oil respectively [10], [12]. This unfavourable result might be due to the effect of

benzene and xylene on the function of bone marrow. Through myelotoxic actions, benzene produces hematologic changes ranging from pancytopenia to total bone marrow aplasia meanwhile; xylene may also cause leukocytopenia [13]. Moreover the decrease TLC was due to the possible reason of their getting used up while encountering a variety of inflammation injury due to toxic agents. This is probably due to the suppression or destruction of myeloid stem cells in the bone marrow, which leads to decrease of leukocyte numbers [14]. The DLC carried out in this study revealed the decrease of neutrophil and eosinophil percentages as compared to control. The susceptibility of bone marrow towards benzene may be a potential cause as mentioned earlier. Benzene causes irreversible damage to myeloid progenitor cells, causing permanent reduction in concentration of erythrocytes, platelets and neutrophils [15]. Antagonistically, the exposure of petrol for 30 days significantly increased the percentages of agranulocytes (lymphocytes and monocytes). Probable, lymphocytosis has been associated by a wide variety of processes including minor viral infection or from multiple petrol chemical exposures [16]. The phenomenon is a complex interplay of petrol constituent exposure (volatile organic solvent, hydrocarbons and others) as revealed by increased of lymphocyte count. It is possible that exposure to petrol constituents may cause an impairment in concomitant stimulation of humoral immunity [16]. In addition, the monocytosis reported in this study, was in agreement of Jorunn *et al*. (2008) although there was no concrete argument on the finding [17]. Nevertheless, overall these studies show that petrol compound such as benzene induces a hematotoxic effect in both myeloid and lymphoid cell lines. Table 3 shows significant decreased in NPA in exposed rats which suggesting petrol exposure has detrimental effect on phagocytosis activity of neutrophils. Neutrophil has similar function with monocyte in phagocytic activity which participating in the destruction of pathogen through phagocytic uptake, intracellular and extracellular enzymatic degradation of toxic substances. A reduction in the number of neutrophils may weaken the ability of the affected cells to phagocytize microbial pathogens and thereby increase their susceptibility to infection [18]. Pervious study showed that crude oil consumption by mallard ducks resulted in lower resistance towards *Pasturella multocida* infection [19]. The reduction of phagocytic activity of cell may due to significant defect in chemotaxis and loss of directionality of neutrophils [20].

5. Conclusion

In conclusion, this study suggests that repeated exposure to petrol vapours for 30 days may elicit hematotoxicity particularly on leucocyte profiles, thereby impairing the normal immune functions. People that occupationally exposed to the petrol vapours therefore require regular medical check-up to ascertain their health condition and wear their personal protective equipment during working hours.

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