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Investigation of the Gasoil Inductive Effects on Blood Parameters of White Albino NMRI Mice

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Background & Aims of the Study: Gasoil is one of most-used oil products applied as diesel fuel, for instance, which, since it consists of aromatic compounds, is among the most important environmental pollutants. Considering its harmful effects on living organisms and human beings, the purpose of the present study was to investigate the effects of gasoil ingestion on blood factors of white Albino NMRI mice.

Materials & Methods: 40 adult mice, with an average weight of 28 grams, were placed in four groups. Adult male mice were placed in two groups, including a control group and a treatment group and adult female mice were divided into two groups of control and treatment. For 14 days, the treatment groups were fed once per day with 0.2cc of pure gasoil solution with a ratio of 6.6 mg/kg of the mice's weight. After anesthesia and blood sample collection, blood parameters, including the number of white and red blood cells, hematocrit and hemoglobin were respectively measured using Neubauer slide, capillary tube, and Sally method. Then, the analyses performed using SPSS19 software.

Results: Variations observed in the blood parameters of male and female mice placed in the treatment groups compared with the control group indicated a significant increase in hematocrit (8%, 10%), a significant decrease in hemoglobin (6%, 10%), and no significant increase in the average number of RBCs (6%, 6%). The level of blood leukocytes consisting of lymphocytes and neutrophils indicated a decrease, while the level of blood. leukocytes consisting of degenerated lymphocytes indicated a significant increase ($P<0.05$).

Conclusions: Due to the increased use of gasoil and generalization of the results of the present research to human beings in terms of leukocyte reduction and weakening of the immune system, hemoglobin reduction and tissue oxygenation disorder, in addition to environmental damages, this substance imposes irrecoverable damages on human health. Hence, necessary measures should be taken by authorities for replacing, reducing its effects and raising public awareness of the methods of dealing with its effects.

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Background

Gasoil, with the chemical formula of $C_n H_2NOS$, is a product of crude oil refineries obtained during the distillation of crude oil, compounding with gas in order for better ignition. Generally, there are four major components of crude oil and gasoil, including paraffin, naphthenes, aromatic and resins-asphaltenes. Iran's crude oil contains high sulfur compounds such as hydrogen sulfide, mercaptans, disulfides, and the like. This type of compounds causes problems likes acid rains and poisoned catalysts (1).

Gasoil and its petroleum products, such as hydrocarbon mixtures, have been studied using degradation and analysis in micro-organisms (2). Diesel engines use gasoil as fuel. This substance is used as a raw substance for refineries to produce petroleum products, including gasoline, and sometimes its highly volatile products are used for washing some parts of machinery and cleaning plant and animal fibers or metal surfaces.

Another stated that the biodegradation of gasoil in 28 days is approximately about 40% (3). Moreover, Mobil respectively stated that the biodegradation of gasoil is 57.5 % (4). In anaerobic conditions, biodegradation of gasoil is negligible.

Due to the stability of oil compounds, their effects on human resources, water and environment varies. Aromatic compounds are among the major environmental pollutants that accumulate in the environment and act as an important factor in environmental destruction. Mutagenic effects of these compounds on human health are also completely unknown (5). Results of gasoil ignition in similar conditions with different percentage of aromatics are different, e.g. the higher the level of aromatics in gasoil, the lower the lethal dose (6).

Regarding their risks to human health, all chemical substances somehow dealing with our daily life should be carefully studied. It is a basic right of any individual to be exactly aware of the hazards of the substance with which he/she deals with. Considering the environmental effects mentioned above, having the knowledge of these compounds, measuring them in air, water and sediments of the operational areas as well as the human body or other living organisms is of high importance (7). More than 3.1 million people in all countries are currently working on the fuel and petroleum section and may be exposed to petroleum products such as gasoline, gasoil and etc. that affect their respiratory and dermal systems. Additionally, millions of other people are exposed to the vapor from the fuels or dusts containing these fuels that can be absorbed into the body by inhalation and can poison the body (8). Due to the use of gasoil as fuel in heavy vehicles, its availability and high use, there is a possibility that its incomplete ignition may increase air pollution. Moreover, due to the evaporation in fuel stations, leaking pipes, transport tankers, oil leakage into groundwater, and the volatility of its aromatics, environmental pollution and toxicity may increase. Using such a substance naturally pollutes the environment and if leakage occurs in soil, water, industrial and organic compounds, the issue should be of interest to other institutions, such as fisheries, environmental protection, health, and food industries.

So far, enough studies have not been conducted about the effects of gasoil on blood parameters of living organisms, especially mice (7, 9-10). The current study attempted to investigate the effects of the ingestion of this substance on the blood parameters in both genders of mice.

Blood is a fluid environment containing cells and plasma, which constitutes 8% of the total weight of the body and constantly another generation of stem cells are produced and replaced in hematopoietic member (11). Plasma and hemoglobin play an important buffer role by keeping blood pH constant (12). The number of RBCs in men and women is 1.4-6 and 3.9-5.5 million mm^3 of blood, respectively. Their life span is about 120 days (13). The concentration of hemoglobin in men and women's blood is 14-18 gr/dl and 12-16 gr/dl, respectively (14). Hemoglobin concentration in different strains of white mice is 10.9-16.3. The RBCs of mice, guinea pigs, rabbits and other mammals are more or less similar to those of humans. Different strains of mice have 6 to 11 million RBCs per mm^3 (15). Hematocrit is the estimation of erythrocyte mass volume per unit of blood volume, which is naturally about 40-50% in men and 35-40% in women (16).

The level of hematocrit is naturally dependent on the number of RBCs, weight, size, age, altitude and other factors (13). The level of various strains of mice is reported about 42% (15). WBCs and platelets constitute less than a percent of the blood volume (17). WBCs are complete cells with a number of 5000 to 9000 per mm3 in normal individuals. This is higher in children and varies highly in the case of diseases (18). Based on the nucleus shape and the type of cytoplasmic granules, leukocytes are divided into two categories: agranulocytes, which contain lymphocytes and monocytes (11), and typical lymphocytes, which approximately have the same size of RBCs and comprise 20-30% of lymphocyte population and are observed in different sizes (19).

Neutrophils are agranulocytes of polymorphonuclear with two to five labial nuclei which are highly active, multiform and are generally the first WBCs which enter the injury (16, 19). The number of WBCs in mice strains varies between 3000 to 14200 mm3 (15). The number of neutrophils, lymphocytes and monocytes varies from 10 to 60, 35 to 85 and 1 to 4%, respectively. Experience has shown that the gender, species, strain, life condition (food, temperature, humidity, season and time of day), hormonal changes and blood collection conditions affect the number of WBCs. In Rollo phenomenon, RBCs form long strands or join laterally which is observed following an increase in fibrinogen and plasma globulins in inflammations and infections. Increased number of peripheral WBCs is generally called leukocytosis, which is caused naturally or physiologically and is associated with peripheral granulocytes moving into the blood flow observed in intense physical activity, anoxia, pregnancy, parturition, and stress (12). In leukocytosis, the number of leukocytes excessively increases. Lymphocytes inflate from stress, toxicity, viral diseases and immune system responses, and their nucleus and cytoplasmic are destroyed and turn into abnormal lymphocytes against viruses or antigenic stimulations (20, 21).

Materials & Methods

This study was conducted on 40 male and female Albino NMRI mice (Pasteur Institute of Iran) (22) with a weight range of 25-30 g in vitro and under appropriate factors which were as follows:

1. Mice were kept in 15 × 30 × 45 cm steel cages.
2. Mice were provided with enough food and water (both control and treatment groups). Their food (plete) was bought from Tehran Pars Animal Feed Company that is a ready-made food. Water bottles were filled with the city's potable water piping system.
3. Cages were cleaned once a week and, if necessary, detergents and disinfectants (dishwashing liquids and 70% ethanol) were used. Food waste was collected daily and fresh food provided again.
4. The temperature was kept at about 22±2° C. Temperature regulation was conducted using a mercury thermometer and an electric heater and a cooler was used to keep the temperature of the room constant.
5. The period of lighting and darkness was regulated in this way: 12h of lighting and 12h of darkness. The light source, in addition to the lighting of the room, was a fluorescent lamp with an exposure time of 7 am to 7 pm.

Experimental Groups

Mice divided into four groups including two control groups and two treatments. Adult male mice were placed in two groups including a control group and a treatment group and adult female mice were placed in two groups of control and treatment with an average weight of 28 grams. For 14 days, the treatment groups were fed once per day with 0.2cc of pure gasoil solution with a ratio of 6.6 mg/kg of the mice's weight (6, 23).

Gasoil is physically liquid which was injected into the experimental groups' stomach with insulin syringes having gavage needles no. 20 purchased from Iran Razi Raad Company using the gavage method. The control group received no such treatment. During the treatment period, the weight of mice was measured every day. To increase the accuracy, gavages were performed at a specific time each day.

Blood Collection, Dilution and Counting Blood Cells

After the end of each period and dissection of the animal, blood collection was performed using a 2-ml syringe that was soaked in EDTA anticoagulant from the right auricle of the heart. To dilute the blood in order to count WBCs, a white melanjor pipette was filled with blood. Then, the melanjor was filled up to 11 degree with Markano solution so that no air bubbles be formed. Afterwards, the pipette was placed in a shaker for 5 minutes to mix the blood and the Markano solution (in this case the blood was diluted with a ratio of 1:10). To dilute the blood in order to count RBCs, a red melanjor pipette was filled with blood up to 0.5 degrees and then was vertically entered in the hyme's dilution. Afterwards, the pipette was filled with the solution up to 101 degrees. In this case, the blood was diluted with a ratio of 1:200. A drop of each melanjor solution was placed on a Neubauer slide. After globules settling, they were counted with a microscope at ×400 magnifications. However, this task can be performed with full automatic Coulter.

Measuring the Percentage of RBC Volume Using Micro-Hematocrit Method

A hematocrit capillary tube was filled with blood up to ¾ of the total volume. The end of the capillary tube was blocked with a special paste. Then, it was centrifuged at 3000 rpm for 15 min., afterwards, the total volume (percent) of RBCs and WBCs to the blood plasma was calculated using a dial (24).

Measuring hemoglobin Using Sally Method

In this method, 20 ml of blood containing EDTA is collected by a Sally pipet and then it was transferred using a hemometer tube which was already filled up to 2 degree with normal HCl 0.1, and was added to distilled water so that the middle tube changed into the same color as the side tubes. Then, the solution level was read and the amount of hemoglobin was determined in grams of the percent of blood solution. The hydrochloric acid solution was hemolyzed red blood cells and release hemoglobin. Mixing with hydrochloric acid, hemoglobin turns into hematin and the intensity of its color depends on the concentration of hemoglobin in the blood. The produced solution was compared with Sally's standard solution (12).

Fruti Preparation and Staining

A drop of blood was placed down on a slide. Using another slide on a 30 ° angle, a consistent range was prepared. After drying and fixing with methylic alcohol, it was stained with Giemsa stain solution. Finally, with a microscope, the differential number of WBCs was counted. Using Markanov solutions (to stain WBCs) and Hyme solution (to stain

RBCs), WBCs and RBCs were counted and photos were taken from the blood samples by a microscope and loop equipped with a digital camera.

Statistical Methods: For the analysis of data obtained from the measurement of blood parameters, one-way analysis of variances was used for the treatment groups (under gasoil stress) and the control groups. To evaluate the within groups differences and comparing mean values, the Tukey's Honestly Significant Different test, as a Post hoc analysis method, was used. Results were expressed as Mean + SEM, and P values lower than 0.05 were determined as significant difference levels. Excel was used to plot graphs.

Results

The average number of RBCs in the male and female mice place in the treatment (stress) groups was 9.6 and 10 million/mm^3, respectively, which compared to the related control group (9 in control females and 9.5 million/mm^3 in control males) indicated no significant difference at the 95% confidence level (P>0.05). Figure 1 illustrates the relationship.

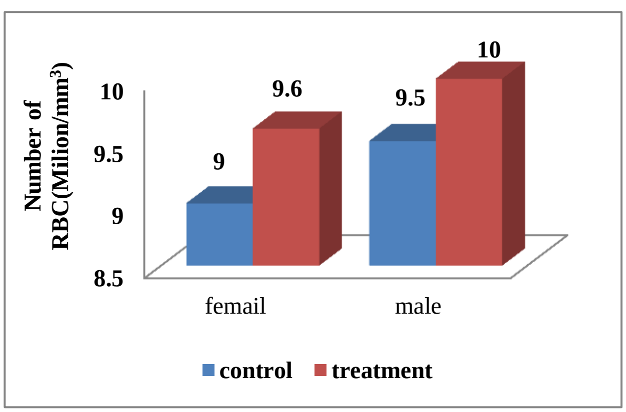

Figure 1) Effect of gasoil on the number of RBCs in the white Albino NMRI male and female mice placed in the treatment groups compared to the control groups (P>0.05)

Investigating the morphology of the blood cells in the male and female mice placed in the treatment groups, various states of physical deformation emerged. The Rollo phenomenon in RBCs, platelets reduction (thrombocytopenia), and degenerated lymphocytes increase in some of the treatment groups' male and female mice were caused by the effects of gasoil in the blood. Figure 2 represents the changes.

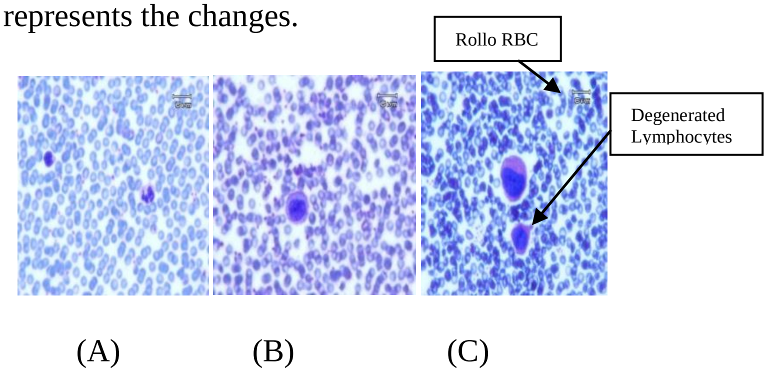

Figure 2) Degenerated lymphocytes and Rollo RBCs in the blood of mice (A) control (B) female mice in the treatment group and (C) male mice in the treatment group (×1000)

The mean volume percent (%) of hematocrit in the male and female mice placed in the treatment groups increased compared to that of the control group. In the female mice in the treatment group, this mean value was 41% which showed a 38% increase. Additionally, in the male mice placed in the treatment group, this mean value was 44% which showed a significant 40% increase (P<0.05).

Measuring the(gram per deciliter)gr/dl of hemoglobin in the blood of treated male and female mice in contrast to a mild increase observed in the amount of RBCs and hematocrit had a significant decrease compared to the control group (P<0.05),

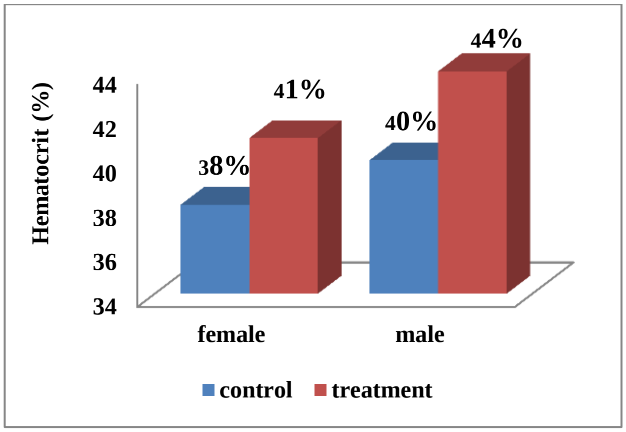

Figure 3) Effect of gasoil on hematocrit of male and female white Albino NMRI mice placed in the treatment groups compared to the control groups (P<0.05).

So that its level in the female treated samples reached 12.5 gr/dl which was lower than 13.3 gr/dl of hemoglobin in the blood of the control mice. In addition, the level of hematocrit in the male treated samples reached 13 gr/dl, which was lower than 14.5 gr/dl of hemoglobin in the blood of the control mice. Figure 4 indicates the changes.

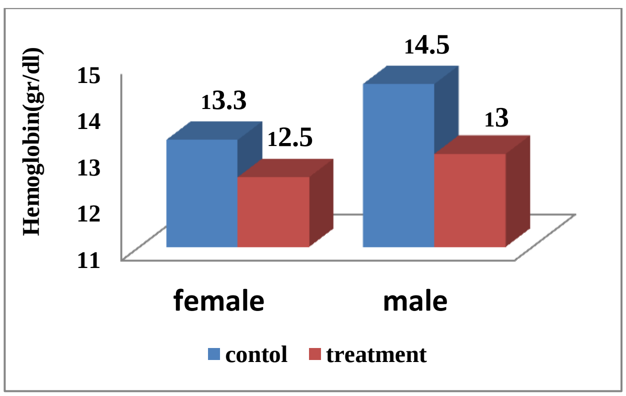

Figure 4) Effect of gasoil on hemoglobin of male and female white Albino NMRI mice placed in the treatment groups compared with the control groups (P<0.05).

Leukocytes in mice blood samples mostly contained small, medium and large lymphocytes and neutrophils and monocytes are hardly seen in normal conditions. Therefore, the number of WBCs includes lymphocytes and neutrophils. Looking at the average number of WBCs in the female and male mice placed in the treatment groups, it was revealed that unlike the mild increase in the number of RBCs, the average number of WBCs decreased in both genders. This was 4500 mm^3 in the female control samples, 3200 in the female treatment samples, 5400 in the male treatment samples and 3300 in the male treatment samples.

Reduced number of the types of lymphocytes and neutrophils, and increased number of degenerated lymphocytes in the male and female samples of the treatment and control groups are shown in Table 1.

Table 1) Effects of gasoline on the types of lymphocytes, neutrophil and degenerated lymphocytes among the white Albino NMRI mice (P<0.05).

Sample	Female control	Female treatment	Male control	Male Treatment
Small lymphocytes	32%	17%	25%	22%
medium lymphocyte	25%	24%	28%	20%
Large lymphocytes	21%	20%	23%	21%
Degenerated lymphocytes	2%	17%	4%	18%
Neutrophils	18%	13%	20%	13%

Discussion

Repeated use of gasoil and distilled oils are associated with skin stimulations, skin tumors and cancers which related to 3-7 poly-cyclic aromatic. The gasoil applied to the skin of male mice for 2, 4 and 7 days a week within 14 weeks induced melanoma skin tumors (melanin generating tumors), fibrosarcoma (tumor of string mesenchymal), and papilloma (6, 25). In general, because of the complexity of the compounds of gasoil, it has a complex toxicology with target organs including the respiratory system, mucous membranes, eyes and the nervous system. The symptoms, which will be observed in a living organism exposed to gasoil, include headache, dizziness, lethargy and malaise, itching, burning and irritation on the skin and in the eyes, runny nose and pain, cough and lung damage (26).

In the male and female mice being treated with different doses of gasoil (500 and 2000 mg/kg), for 5 days a week during 13 weeks, skin irritation and death of animals tested were reported. Carcinogenic characteristics of gasoil have been also studied (7). Skin stress and edible gasoil (65% to 79% ethylene) produced different results (27, 28).

According to Figure 1, the average number of RBCs in the female and male mice placed in the treatment group compared with that of the control group increased about 6%. The one-way analysis of variance revealed that this difference was not significant ($P<0.5$). In the morphology of the blood cells in the male and female mice placed in the treatment groups, various states of physical deformation were observed. Anisocytosis observed in most mice of the treatment group indicated the presence of small (microcytosis) and large (macrocytosis) blood cells that occur together.

Rollo appearance, decreased platelets in some cases, and contrastingly formation of megakaryocytic cells and platelets in peripheral blood or platelet aggregation in other cases of male or female mice in the treatment groups were among the effects of gasoil which were observed in samples of blood smear. The mean volume percentage of hematocrit according to Figure 3 increased 10% and 8% in the male and female mice in the treatment groups, respectively, compared to that in the control samples. This significant relationship may be due to addition of RBCs and reduction of water of the blood; thus, increased RBCs have increased hematocrit which is consistent with previous findings (29, 30).

In Figure 4, it was observed that measuring the gr/dl of hemoglobin in the male and female treatment mice, compared with that in the control samples, in contrast with the mild increase obtained in the number of RBCs and hematocrit, a significant decrease was observed. The level decreased by 6% in the female mice placed in the treatment group and 10% in the male mice placed in the treatment group.

Of course, in the natural state measuring the gr/dl of hemoglobin the blood of mice, this is 10.9-16.3 gr/dl (15).

Usually, the increase of the number of RBCs and hematocrit is associated with the increase in the number of hemoglobin in RBCs which relate to hemoglobin concentration as well as dehydration (29).

The number of WBCs in mice strains varies from 3000 to 14200 per mm^3 (15).

Leukocytes in the blood samples of rats mostly contained small, medium and large lymphocytes and neutrophils and monocytes are hardly seen in normal conditions. Therefore, the number of WBCs includes lymphocytes and neutrophils shown in Table 1. The average number of WBCs in the male and female mice in the treatment groups, in spite of a mild increase in the number of RBCs, decreased in both genders. The reduction was 30% in the female mice placed in the treatment group and 40% in the male mice in the treatment group per mm3 of blood which is consistent with the study in which gasoil with a dose of 500 mg per body weight in 13 weeks reduced the number of lymphocyte. In the present research, the reduction of lymphocytes in the males was higher than that in the females (30).

The increased percentage of the number of degenerated and damaged lymphocytes in the blood of mice exposed to treatment was evidence of the deleterious effects of gasoil on WBCs. In the current study, the number of degenerated and damaged blood cells in mice increased because lymphocytes were swollen during stress and toxicity, and their cytoplasm and nucleus were destroyed (21).

The decrease in WBCs in the more or less presence of precursor cells, such as lymphoblasts and band cells, was associated with the appearance of leukocyte cells, such as plasma cells, Ryder cells, and etc. Ancestral cells, such as lymphoblast, monoblast, and plasmablast cells, were observed in peripheral blood with 4 and 6 percent in the female and male mice, respectively. However, the results of Mobill study in which gasoil (9.56% ethylene) was rubbed on the skin of male and female mice at doses of 30, 125, 500, 2000 mg per body weight in 13 weeks and increased number of WBCs was observed (4) is not in line with the results of the present research study.(Table 1)

In normal circumstances, usually leukocytosis (increased number of WBCs) is the result of stressors (gasoline) (12). However, causes of the reduction in the number of leukocytes was certainly the result of degeneration or destruction of WBCs, especially of lymphocytes, since the lymphocytes degeneration of the blood of the female and male mice placed in the control groups was hardly 2% and 4%, respectively, while this was 18% and 17% in the female and male treatment groups. The number of neutrophils was also decreased significantly in the treatment groups

Given the inductive effects of gasoil on Albino NMRI mice's blood parameters, the results can be generalized to human beings. Given the high use of gasoil in industries and vehicles as well as its harmful effects on the reduction of leukocytes and in general weakening the immune system, decreasing hemoglobin and creating impaired tissue oxygenation, the presence of this substance can seriously threaten human health in the long term. The following actions should be regarded to avoid such risks:

1. Using compounds together with gasoil to reduce its harmful effects
2. Helping to create a useful plan to conduct further toxicological studies on gasoil
3. Recognizing disorders caused by gasoline ingestion or by its inhalation which are in connection with changes in various organs and tissues of rats
4. Observing precautions when handling this substance at petrol stations and refineries; and
5. Informing the public about the hazards of this substance and providing strategies to reduce the resulting complications

Footnotes

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Conflict of Interest:
The authors declare no conflict of interest.

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