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Effect of Exposure to Inhalation of Selected Petroleum Products on Liver function of Male Albino Rats: A Comparative Study

Chinedu Imo[1], Friday O. Uhegbu[1] and Ifeanacho Nkeiruka Glory[2]
[1]*Department of Biochemistry, Abia State University, Uturu, Abia State, Nigeria.*
[2]*Department of Animal Production and Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.*

Abstract: *This study examined the comparative effect of exposure to inhalation of Kerosene, Diesel, Petrol and a mixture of kerosene, diesel and petrol on liver function of male albino rats. The serum liver enzymes ALT, AST and ALP increased in all the test animals exposed to inhalation of the petroleum products when compared to the control. ALT increased significantly ($p \leq 0.05$) in group 4 (from 36.60 ± 2.41 to 41.80 ± 1.64 IU/L), but increased non-significantly in groups 2, 3 and 5 compared with the control (group 1). AST increased significantly ($p \leq 0.05$) in all the groups compared to the control with highest increase in the group exposed to diesel (from 17.40 ± 2.51 to 24.40 ± 3.71 IU/L). ALP also increased significantly ($p \leq 0.05$) in all the test animals compared to the control with highest increase in group 3 (from 27.92 ± 0.16 to 44.93 ± 0.87 IU/L). Total bilirubin increased significantly ($p \leq 0.05$) in all groups exposed to the petroleum products with the highest increase in group 4 (from 0.20 ± 0.03 to 0.51 ± 0.05 mg/dl) when compared with the control. Haematological analysis show a significant ($p \leq 0.05$) reduction in WBC levels in groups 2, 3 and 5, and a non-significant reduction in group 4 compared with the control. RBC increased significantly ($p \leq 0.05$) only in group 3 (from 6.39 ± 0.88 to 7.76 ± 0.37 $x10^{12}$/L) and increased non-significantly in groups 2, 4 and 5. Hb increased non-significantly ($p \leq 0.05$) in group 2 and 3, and decreased non-significantly in group 4 and 5 compared with the control (group 1). PCV increased non-significantly ($p \leq 0.05$) in groups 2 and 3 (from 41.31 ± 5.94 to 41.98 ± 5.47 % and 41.31 ± 5.94 to 45.57 ± 5.11 % respectively), but reduced non-significantly in groups 4 and 5 compared to the control. Histological analysis of Liver section from rat in group one (normal control) show essentially normal histoarchitecture of the liver tissue, but exposure of the animals to inhalation of the petroleum products show distorted arrangement or alterations of the normal histoarchitecture of the liver sections compared with the control. The results of this study show that exposure of the albino rats to inhalation of petroleum products can cause slight alteration in haematological parameters, but can cause significant alteration in levels of liver function parameters and distortion in normal histoarchitecture of the liver tissue and therefore, can cause hepatic damage.*

Keywords: *Diesel, Inhalation, Kerosene, Liver, Petrol.*

I. Introduction

Crude petroleum is a mixture of different hydrocarbons and metals [1]. The chemical composition of crude petroleum varies between geographical locations. Crude oil is refined into fractions of kerosene, diesel, petrol, heavy gas oils, lubricating oils, as well as residual and heavy fuels, among others. Kerosene, diesel and petrol are the most commonly used fractionated crude petroleum products. Petrol contains aliphatic, aromatic and a variety of other branched saturated and unsaturated hydrocarbons at variable concentrations [2, 3]. Studies have reported the haematotoxic [4] and hepatotoxic, [5] effects of hydrocarbons. The constituents of the vapours from different fractions of petroleum products depend on the composition of their liquid forms, which varies with the brand and storage period.

Gasoline, kerosene and diesel are reported to contain predominantly, hydrocarbons with carbon atoms 4-10, 11-13 and 14-18, respectively [6]. The volatility of these fractions varies with the predominant hydrocarbon species. Unleaded gasoline for instance, is reported to contain about 300 different hydrocarbon species, most of which are highly volatile and may evaporate if left exposed, to constitute ubiquitous chemical pollutants in the environment [7].

Exposure to various fractionated products of crude petroleum has been reported to cause impairment of renal function evident by the derangement of serum electrolytes [8, 9]. During the course of usage of these products, individuals are usually exposed to pollutants from petroleum products in their environments. Those that are occupationally exposed tend to be at a greater risk of exposure [10] to the petroleum products. Petrol is used as fuel for motor vehicles, generators and power plants. It is also used as pesticides and cleaning agent. The chemical pollutants from petrol vapours, like other known xenobiotics, may be metabolically transformed into various metabolites in the body [11]. Some of these metabolites may be very reactive, interacting in various ways with the metabolizing and excreting tissues (mainly the liver and kidneys) to elicit toxic effects [12]. The

interaction of these metabolites with the tissues may cause damage to the tissues. Hydrocarbons like benzene, metals like lead and volatile nitrates have been reported to produce harmful effects on bone marrow, spleen and lymph nodes [13]. These toxic compounds destroy or inhibits the hematopoietic stem cell in the bone marrow [14, 15]. Benzene which is an aromatic hydrocarbon contained in petroleum product is known to induce leukemia during occupational exposure [16].

Metabolism of poisonous chemical substances takes place in the liver, which accounts for the organ's susceptibility to metabolic induced hepatotoxicity. The crude oil and its products generate free radicals and promote a variety of chemical reactions, such as depletion of reduced glutathiones or inducing lipid peroxidation. Sudakov [17] showed that increased levels of lipid peroxidation are indicative of severe liver, kidney and heart damage such as acute myocardial infarction and artherosclerotic plaque stability. Despite the adverse effects of some petroleum products, it has been employed therapeutically for medical purposes mostly in rural areas.

II. Materials And Methods

2.1 Petroleum Products

Petrol, kerosene and diesel were purchased from a Petrol station in Uturu, Abia State, Nigeria.

2.2 Experimental Animals

Twenty five male albino rats aged 7 weeks (between 130g-160g body weight) were used in this study. The rats were bought and kept in the animal house, Department of Biochemistry, Faculty of Biological and Physical Science, Abia State University, Uturu. The animals were allowed to acclimatize for 7 days under standard laboratory conditions with free access to commercial rat feed and water.

2.3 Experimental Design

The twenty five animals were randomly placed into five (5) groups with five (5) rats in each group. Group 1 served as the control group (it was not exposed to any Petroleum product). Group 2 was exposed to inhalation of Kerosene. Group 3 was exposed to inhalation of Diesel. Group 4 was exposed to inhalation of Petrol. Group 5 was exposed to inhalation of a mixture (equal volumes) of Kerosene, Diesel and Petrol. The five different groups were kept far from the location of one another.

Groups 2, 3, 4 and 5 were exposed to the products (as stated above) five hours daily for twenty one (21) consecutive days. During the five hour daily exposure, the products were placed in plates (without cover) and stationed very close to the cages of the animals constantly and also occasionally sprayed around the environment where the animals were stationed. All animals were allowed free access to feed and water *ad libitum*. Standard laboratory protocols for animal studies were maintained.

The essence of exposing the animals to the petroleum products for five hours daily is to accommodate the fact that most workers in standard or well-established petrol stations work for about four (4) to five (5) hours daily, though some also work up to eight hours daily. The reason for the occasional spray around the environment where the rats were stationed is to accommodate the fact that occasionally at the petrol stations, some of the products may unknowingly be sprayed within the station as a result of force out of a container under pressure or over-filling of the tanks, cans, etc.

2.4 Blood Collection

Twenty four hours after exposing the animals to inhalation of the petroleum products, the animals were starved overnight, anaesthetized with chloroform and sacrificed. Blood was collected by cardiac puncture and blood samples from each animal collected into dry test tubes. The blood sample was divided into two. The first part was dispensed in heparinized tubes for haematological analysis, while the second part of the blood sample was allowed to stand for about 15 minutes to clot and further spun in a centrifuge. Serum was separated from the clot with Pasteur pipette into sterile sample tubes for the measurement of selected liver function parameters.

2.5 Biochemical Analysis

The serum concentrations of Aspartate transaminase (AST) and Alanine transaminase (ALT) were determined using the colourimetric method described by Schmidt and Schmidt [18]. Alkaline phosphatase (ALP) was determined using the method described by Tietz [19], while Total bilirubin was determined according to the method of Sherlock [20].

Haematological parameters: Red blood cell, White blood cell, Haemoglobin and Packed Cell Volume were analyzed using haematological auto-analizer (Abacus 380).

2.6 Histological Analysis

After sacrificing the animals, histological analysis was carried out on the liver of representatives of each of the five groups.

2.7 Statistical Analysis

The results were subjected to statistical analysis using Analysis of Variance (ANOVA) and standard student-T-distribution-test: using Statistical package for Social Sciences (SPSS) version 20. Group means were compared for significance at $p \leq 0.05$. Data were represented as mean ± standard deviation.

III. Results

The results are as presented in the tables and plates below

Table 1: Liver Enzymes and Bilirubin Concentration

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
ALT (IU/L)	36.60 ± 2.41^{a}	38.20 ± 1.79^{a}	36.80 ± 2.05^{a}	41.80 ± 1.64^{b}	41.00 ± 1.87^{a}
AST (IU/L)	17.40 ± 2.51^{a}	22.80 ± 2.49^{b}	24.40 ± 3.71^{c}	21.80 ± 2.17^{d}	22.00 ± 2.21^{e}
ALP (IU/L)	27.92 ± 0.16^{a}	43.50 ± 2.01^{b}	44.93 ± 0.87^{c}	38.74 ± 1.05^{d}	33.85 ± 1.77^{e}
TOTAL BILIRUBIN (mg/dl)	0.20 ± 0.03^{a}	0.34 ± 0.05^{b}	0.38 ± 0.04^{c}	0.51 ± 0.05^{d}	0.39 ± 0.04^{e}

Results represent mean ± standard deviation of group serum results obtained (n=5).

Mean in the same row, having different letters of the alphabet are statistically significant ($p \leq 0.05$) compared with the control (group one).

LEGEND: ALT= Alanine Aminotransferase, AST= Aspartate Aminotransferase, ALP= Alkaline Phosphatase and IU/L= International Unit per liter.

Table 2: Concentrations of Haematological Parameters

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
WBC ($X10^{9}$/L)	11.86 ± 1.34^{a}	8.53 ± 1.83^{b}	9.98 ± 0.16^{c}	11.32 ± 1.07^{a}	10.04 ± 0.17^{d}
RBC ($X10^{12}$/L)	6.39 ± 0.88^{a}	6.57 ± 1.02^{a}	7.76 ± 0.37^{b}	6.47 ± 0.89^{a}	6.42 ± 0.48^{a}
Hb (g/dl)	12.70 ± 1.67^{a}	12.98 ± 1.66^{a}	13.74 ± 1.02^{a}	12.32 ± 1.86^{a}	11.40 ± 1.40^{a}
PCV (%)	41.31 ± 5.94^{a}	41.98 ± 5.47^{a}	45.57 ± 5.11^{a}	39.76 ± 6.94^{a}	37.15 ± 2.77^{a}

Results represent mean ± standard deviation of group results obtained (n=5).

Mean in the same row, having different letters of the alphabet are statistically significant ($p \leq 0.05$) compared with the control (group 1).

LEGEND: WBC= White blood cell, RBC= Red blood cell, Hb= Heamoglobin, PVC= Packet cell volume, %= percent and g/dl= grams per deciliter.

Histological results

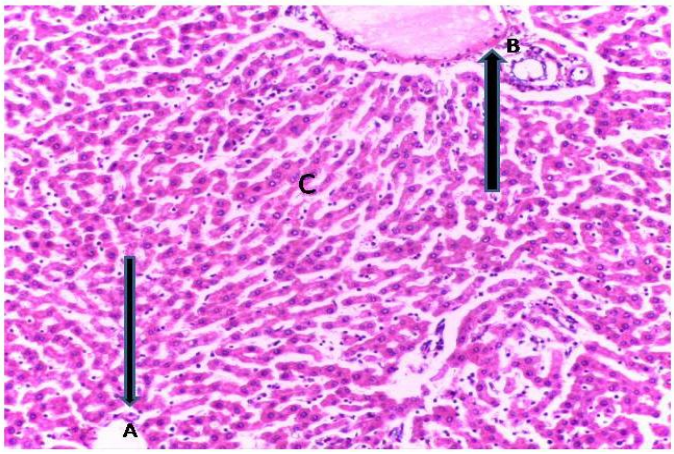


Plate 1: Liver section photomicrograph from rat in control group (group 1)

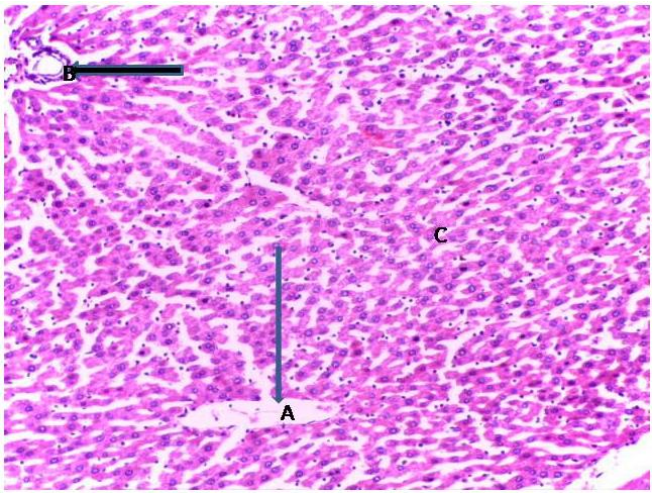


Plate 2: Liver section photomicrograph from rat exposed to Kerosene (group 2)

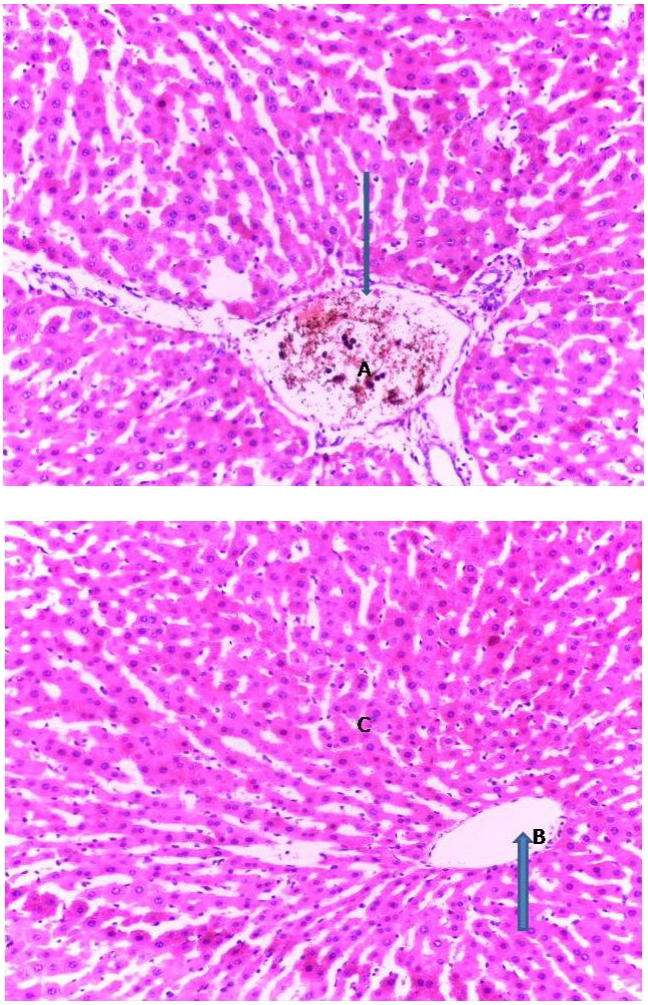


Plate 3: Liver section photomicrographs from rat exposed to Diesel (group 3)

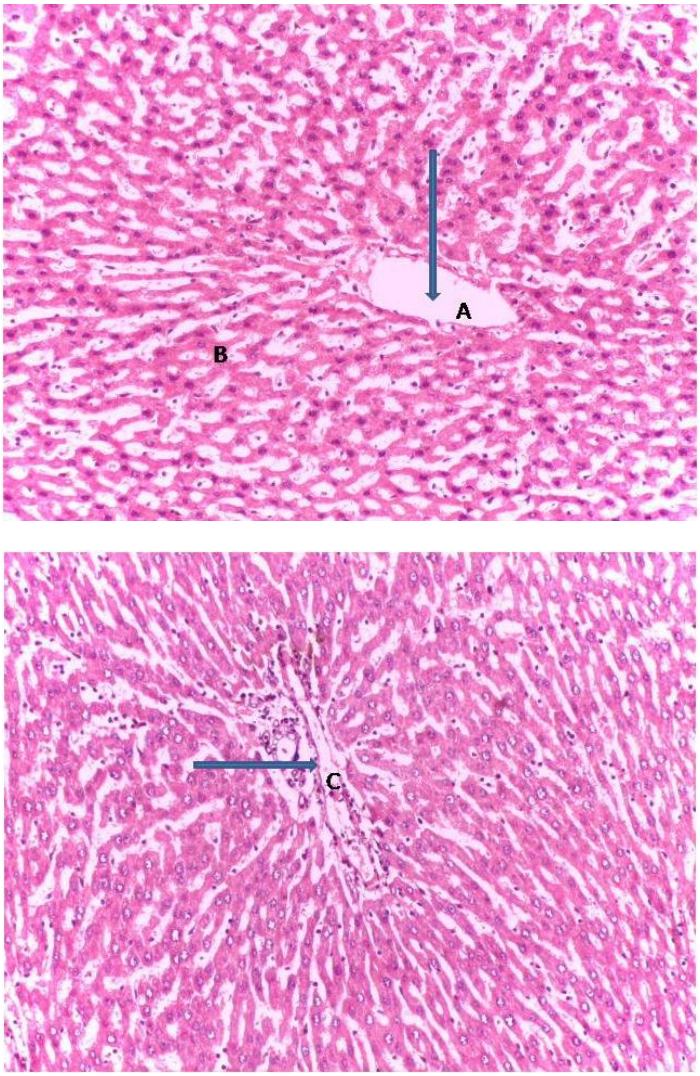
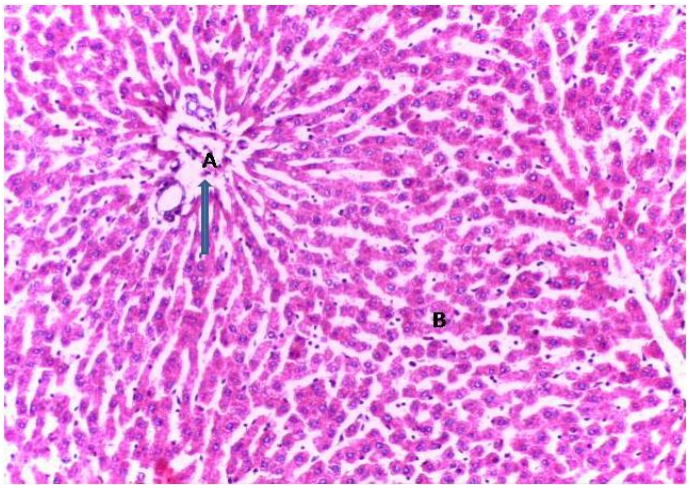


Plate 4: Liver section photomicrographs from rat exposed to Petrol (group 4)



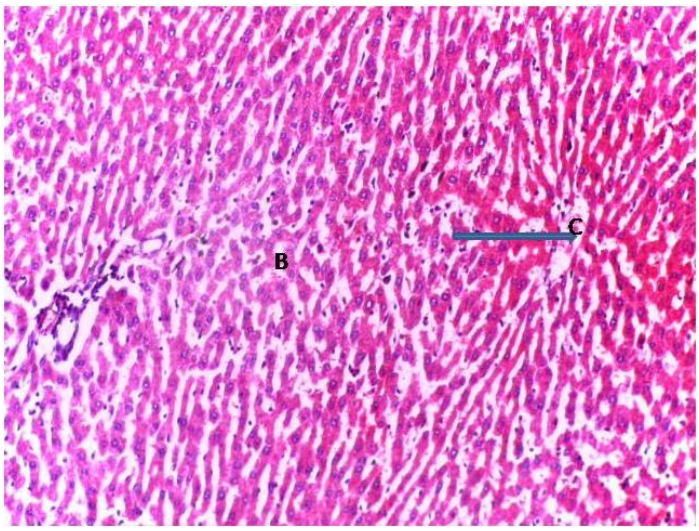


Plate 5: Liver section photomicrographs from rat exposed to mixture of Kerosene, Diesel and Petrol (group 5).

IV. Discussion

The results of AST and ALP of the control animal showed a significant increased ($p<0.05$) after exposure to inhalation of the different petroleum products, while ALT increased significantly ($p<0.05$) only in group 4 (exposed to petrol) and increased non-significantly in groups 2, 3 and 5. The increase in the levels of ALT, AST and ALP shows hepatic toxicity and possible damage caused by the exposure to the petroleum products. Petrol, kerosene and diesel are used for various reasons by humans at home, petrochemical and manufacturing industries. Exposure to petroleum products have been reported to have toxic effects on various organs and systems of the body, resulting in various diseases and different forms of genotoxic, mutagenic, immunotoxic, carcinogenic and neurotoxic manifestations [3, 21].

Liver is an organ involved in many metabolic functions and is prone to xenobiotic injury because of its central role in xenobiotic metabolism [22]. Hepatotoxic substances can cause damage to the liver. The elevated levels of serum enzymes as observed in the groups exposed to inhalation of the petroleum products are significance of cellular leakage and loss of functional integrity of cell membrane in liver. Observation of the effect of the inhalation of the petroleum products to liver function parameters and liver histology of albino rats may give an idea of possible effects of these products on human health (using albino rat model).

Comparative effects of these petroleum products show that AST and ALP levels were altered more by inhalation of diesel, followed by kerosene. ALT and total bilirubin levels were significantly ($p<0.05$) altered (elevated) more by inhalation of petrol, followed by the mixture of petrol, kerosene and diesel.

The significant ($P<0.05$) elevation of total bilirubin levels in all the groups exposed to the petroleum products (group 2, 3, 4 and 5) when compared with the control (group 1) may be associated with reduced hepatocyte uptake of bilirubin, impaired conjugation of bilirubin and reduced hepatocyte secretion of bilirubin. It may also be attributed to obstruction in the flow of bile from the bile duct as a result of severe liver damage or may also be as a result of liver necrosis which can cause the liver not to conjugate bilirubin and cause the hepatocytes to lose its ability to take up bilirubin.

Haematological studies showed a significant ($p<0.05$) decrease in white blood cells (WBC) in all groups exposed to the petroleum products, except in group 4 (exposed to petrol) which reduced non-significantly. Red blood cells (RBC) increased significantly in the group exposed to diesel, but increased non-significantly in the other groups. Packet cell volume and haemoglobin concentration increased non-significantly in groups 2 and 3, but reduced non-significantly in groups 4 and 5 compared with the control (group 1). The low level of PCV and Hb in group 4 and 5 when compared with the control (group 1) may be attributed to the increase in ALT and AST. Increased ALT and AST are indicative of liver damage. The level of white blood cells (WBC) is usually used as an index of immune function and there was significant decrease ($p<0.05$) in WBC levels in group 2, 3 and 5 when compared with the control. It is possible that the inhalation of the petroleum products may have possibly influenced the processes involved in the production of white blood cells. The haematological alterations (though non-significant ($p<0.05$) in most of the parameters) observed seemed not to be very severe. This could be as a result of withdrawal of the animals from exposure to the petroleum products after the five hour daily exposure. It is possible that the body system of the animals tries to adjust during the withdrawal period.

Photomicrographs of sections of Liver from rat in group one (control) show essentially normal histoarchitecture of the liver tissue. Histological sections of rats in group 2 show liver tissue with distorted

arrangement of laminae plate and hepatic triad that seem dilated (B) with spindle shaped slightly enlarged central vein (A). Most of the hepatocytes exhibit hyperchomatic nuclei and some with hollow nuclei (C). Histological sections of rats in group 3 show liver remarkable necrosis within the dilated hepatic triad (A). The stroma appear densely fibrous with some hepatocytes showing hypochromatic nuclei (C). The central vein is enlarged and lined by simple single layer epithelial cells (B). Histological sections of the liver of rats in group 4 show slightly enlarged central vein(A) with multicystic spaces within which are seen pyknotic hepatocytes (B). The hepatic triad is slightly dilated (C). Also seen are numerous ghost cells (ballooning) within the stroma (B), while histological sections of the liver of rats in group 5 show normal hepatic triad (A) and well defined laminae plate lined by slightly enlarged hepatocytes (B). The central vein appears normal though spidle shaped (C). Within the stroma are seen some slightly enlarged hepatocytes against a hyalinized background (B). (10)

V. Conclusion

The results of this study demonstrate that exposure to the inhalation of petroleum products (kerosene, diesel and petrol) could be dangerous to the liver function of male albino rats and may possibly affect human health. Therefore, adequate precautionary measures should be taken (especially by those who use these products regularly) during the processing methods or use of these products to avoid its inhalation. This will help in avoiding the alterations observed in this study.

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