Studying the Histopathological Effects of Styrene on Liver in Rats

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ABSTRACT

Introduction and Objective: styrene is one kind of organic aromatic solvents released during thermal polymerization of styrene in plastics industry. The present study investigates the histopathological effects of styrene on liver in rats. Materials and Methods: the present empirical research was conducted during 2012-2013 in Zahedan's Medical Sciences University on 20 rats assigned to two groups, namely experimental group (styrene exposure) and control (unexposed). The sampling was undertaken in a random manner. In the end, the hepatic tissue changes were evaluated in the experimental and control groups' subjects. Data were analyzed using ANOVA test. Results: the weight rate of the experimental group subjects was 10.51±1.18 and it was 7.62±1.44 for the evidence group. The weight differences between the two groups were not significant (P>0.05). Substantial degenerescence and cell changes were observed in Zone III of hepatic acinus. Disarraying of the hepatic cellular ropes was evident in the experimental group and necrosis and apoptosis traces were visible in the periphery of central venule. Hydropic degeneration along with cell inflammation, nucleus swelling and eosinophilic cytoplasm reduction was amongst the results found for the experimental group.Conclusion: the present study findings indicated that styrene exerts toxic effects on the livers of the rats and causes cell damage and disordering of the hepatic performance.

Key Words: Styrene, Liver, Hepatic Disorders, Rats.

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INTRODUCTION

As a large gland attached to the digestive tract, liver fulfills numerous duties amongst which blood glucose regulation, plasma protein generation, bile acids' secretion and poisonous substances and drugs' neutralization can be pointed out as but some of its function. The liver's being situated on the digestive system's route of blood collection provides for serious damages thereto during its blood cleaning of materials adsorbed in intestines [1]. Hepatocytes are the main cells of the hepatic tissue. These cells account for about 80% of the hepatic tissue. The main functions of hepatocytes are: synthesis of such proteins as albumin, prothrombin, fibrinogen and heparin, storage of protein, metabolism of carbohydrates, production of cholesterol, bile acids and lipoproteins, detoxification, conversion and expelling of external and internal poisonous materials [2]. The damage to the hepatic cells subsequent to the effect of the toxic materials leads to the change in the serum levels of aminotransferases. Although the increase in the amounts of these enzymes is a nonspecific, the intensive increase in these enzymes is always indicative of hepatic cell damages. Metal boiling in industrial processes is an almost non-eliminable process in light and heavy industries and it is known to result in the emission of the gases and aerosols the harmful effects of which on the respiratory system, nerves, reproduction system, eyes and ears have been documented. Also, the change in the levels of sugar ingredients (glycoconjugates) in epithelial cells of bile ducts and Kupffer cells of liver along with the change in their nature has also been demonstrated [1].

Styrene is an important industrial chemical substance that is widely applied for the production of plastics and other polymers like polystyrene, resin, rubber and dyes.

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Exposure to styrene can also be seen due to its existence in automobiles' exhaust and packed food and water [3]. Styrene is a liquid featuring a molecular weight equal to 104.4, a specific weight equal to 0.9g/cm^3 and melting point of 30.6C and boiling point of 145.2C. Styrene's vapor pressure in 25°C is equal to 6.45mmHg. Styrene is a fatty organic liquid featuring aromatic properties and different commercial names like vinyl benzene, phenylethylene, cynamin, diarex, styrlene, styropole and anamene [4]. Styrene is a colorless liquid that is vaporized easily and disperses a sweet odor. There are impurities along with styrene that render its odor unpleasant and pungent [4, 5]. Inhaling large deals of styrene vapor in a short time causes nervous system effects like depression, concentration problems, muscle weakness, fatigue, nausea and nose, eye and throat irritations. Breathing in of the styrene vapors in short-term by the animals causes damages to the nasal mucosa and hepatic symptoms appear in long-term. There are no proofs of such effects on the humans [6]. Liver is the most substantial locus of this chemical compound's metabolism which firstly turns it into styrene oxide and then to other products to be expelled. Hippuric acid is the most common form of styrene excretion [7].

There are evidences proving hepatic cell necrosis by styrene in rats exposed to high amounts (over 300ppm) of styrene through pathological and enzyme evaluations of liver. Hepatic enzyme measurement, though not a precise sign of hepatic performance, is a very important index indicating a great many of the hepatic lesions [1].

It was found out in the study by Bowen et al (2009) that the optimal use of this very important solvent can reduce the contamination concentration thereof in the work and house environments to a large extent thereby to decrease its harmful effects. Also, the toxic effect of the solvent on the foetus was evaluated similar to fetal alcohol syndrome in the study [8]. It was made clear in the study performed by Kishi et al in 2005 on the gestation and gender effects of styrene metabolism in rats' liver that pregnancy negatively influences p450 cytochrome during its final stages. Also, pregnancy was found considerably reducing the formation of glycol styrene [9].

ACGIH has determined bearable styrene threshold in respiratory air equal to 100ppm which is equivalent to 420 mg/m^3. In the study carried out by Leibman in 1975, it was shown that the most major path for styrene's entry of the body is through respiration and/or skin. Styrene is transformed to hippuric acid in the kidneys and liver. The renal and hepatic tissue toxicity by styrene along with weight increases in these two organs has been reported subsequent to oral consumption thereof. On the other hand, according to high solubility of styrene in fats, it enjoys a high rate of absorption through skin. The excretion of mandelic acid and phenyl glyoxal acid is considered as an index of intoxication by styrene in humans. Tissue toxicity by the styrene urinary metabolites has been less frequently reported than styrene toxicity [10]. The studies by Ohlson et al in 2000 indicated that the environmental contaminants can disrupt the hormonal balance in the individuals and cause numerous disorders in the tissues and they can even set the ground for malignant changes in the body organs [11]. In the studies by Kitamura et al in 2003, it was concluded in regard of the activity of styrene oligomers experimentation after hepatic microsomes' activation of metabolites in rats that estrogenic activity of trans-1,2-diphenylcyclobutane (tcb) is created following the formation of 4-hydroxylase metabolite [12].

In the study by Aminian et al in 2003, the effect of chronic respiratory contact with mild to intermediate amounts of styrene on liver performance was investigated in workers who used styrene polymers in plastic part production industry. The case group was comprised of individuals who had been working for more than a year in heavy plastic injection section. To investigate the effects within the format of a cross-sectional study, the levels of hepatic enzyme, including transaminase (ALT-AST), hepatic cholestasis tests (ALP-GGT) and hepatic clearance evaluations (total bilirubin and conjugates) were compared in 58 workers from case group with 52 workers from evidence group. The results of the study are indicative of the idea that chronic contact with low amounts of styrene in plastic injection industry can cause mild cellular effects and hepatic clearance disorders. Therefore, periodical evaluations of hepatic performance were recommended to the individuals working in plastic injection industry [13]. The present study deals with the histopathological effects of styrene on liver in rats.

STUDY METHOD

The present study is an empirical research. The sampling was carried out based on convenience method. The study inclusion criterion was complete healthiness and the study exclusion criterion was the death of the laboratory animal. To perform the research following the accomplishment of preliminary studies, at first, the styrene vaporizer device was installed and styrene vapors were injected into the gas chamber for measuring the required indicators to adjust discharge rate and air ventilation speed so that the amounts of the intended contaminant (styrene) can be stabilized in the exposure cabin. Next, 20 Sprague Dawley rats were procured from Pastor Institute and transferred to animal house of the university and were accustomed to the standard conditions (free access to foodstuff and water, 12/12 light-dark cycle, 22-24°C temperature (Microtherm, Casellacel England), 45-50% moisture (Standard, ST625 Taiwan), proper ventilation

(10-12 times per hour in animal room). The rats with initial mean weight of 200±13 and three months of age were assigned to two experimental and evidence groups (styrene groups with 10 rats (Merck) and control group with 10 rats). The evidence group contained animals that did not have to be exposed to styrene contaminants. The experimental group rats were exposed to styrene vapors in cabin four hours every day (7-11 AM) for three weeks (six days a week). The exposure conditions were kept constant for the experimental group subjects during the experiment and the amount of styrene (purchased from German Merck Company) extant in the cabin air was measured using standard methods in an hourly basis (Phocheck 5000, Ion Science England). The ventilation speed of the air inside the room was set on 1.75 times per hour with the regulation of a spiral fan and installation of an appropriate extractor hood in the gas chamber. The volume of the air inside the cabin was 0.15 cubic meter. The temperature inside the gas chamber was kept constant (24°C), as well. The total amount of styrene used during the experiment was 1850 ml. Rats' weights were measured before and after experiment. The technical specifications of the gas chamber and the pumps (SKC, 224-44Tx, England) used was 0.55×0.5×0.5m. Styrene was poured inside impinge (gas wash bottle). The right-hand side pump created styrene gas through blowing and the styrene gas was sent to the injector. The second pump injected pure air into the injector. The concentration of the gas was measured in the injector outlet (1300 to 1500ppm). The third pump featuring a discharge rate equal to the sum of the other two pumps' discharge rates performed suction to replace the air. The outflowing gas concentration was measured in the chamber outlet and there were two spots on the fourfold surface of the chamber for measuring and sampling the concentration inside the cabin. These measurement spots were only opened for performing measurements and sampling. In the beginning of the exposure, a high concentration of styrene was injected into the cabin and it was rendered even through the use of three fans installed inside the chamber after 15 minutes. After the concentration was found reached 1300-1500ppm inside the chamber, the inflow rate was reduced to 1300-1500ppm using two pumps installed half way to the injector which was kept constant during exposure through regulating the discharge rates and temperatures.

The temperature inside the chamber was recorded using a thermometer the expansion chamber of which was inside the cabin. When the concentration was fixed, the total discharge rate of the two blowing pumps was 4l/m and the suction pumps performed their action in the same discharge rate. The air replacement inside the chamber was carried out according to the dimensions of the chamber (0.55×0.5×0.5m) and a discharge rate equal to 1.75 times per hour in the outlet. Based on the specified time table, experimental and evidence group rats were sampled of their livers after putting them into deep anesthesia using chloroform that resulted in the animals' death. The samples were passed through 1% saline formalin corresponding to the methods common in histology and paraffin blocks were prepared of the sampled tissues. Liver weights in the experimental and evidence groups were measured after fixation. Incisions, 5-7 micrometer in thickness, were made using rotary Micorotome (Leica) and stained using hematoxylin and eosin and Periodic Acid Schiff (PAS) method. The incisions were mounted based on common histological methods and subjected to microscopic studies. To perform quantitative measurements, Zeiss KF2 microscope with micrometer ruler (Zeiss) and all the measurements were recorded using x40 objective lens. The obtained information was analyzed using SPSS and the required graphs were drawn in Excel. LSD-assisted multiway variance analysis (ANOVA) was the method of information evaluation. The significance level was set at $P<0.007$ in all of the analysis. Leica digital microscope was applied for photomicrography. The mean styrene concentration in the air inside the exposure chamber was kept fixed at 1320±140 during the experiment period. PAS and hematoxylin-eosin staining were routinely conducted in histology laboratories. Blood samples were directly collected from the heart and enzyme activity assays were conducted corresponding to the methods common in diagnostic laboratories.

STUDY FINDINGS

In the present study, a total number of 20 rats, assigned to two experimental and evidence groups, were examined. The weights of the experiment group rats were 195±14 before exposure and 213.4±9.4 after exposure. The weights of the evidence group rates were 171±8 before exposure and 189.6±10 afterwards. The rats' changes in weight were not found statistically significant before and after experiment (exposure to styrene) between the experiment and evidence groups ($P>0.05$) (table 1 and diagram 1).

Table 1: comparing the weights of rats before and after styrene exposure in experimental and evidence groups using ANOVA test

Variable	Experiment	Evidence	P-value
Weight before exposure	195±14	171±8	P>0.05
Weight after exposure	213.4±9.4	189.6±10	P>0.05

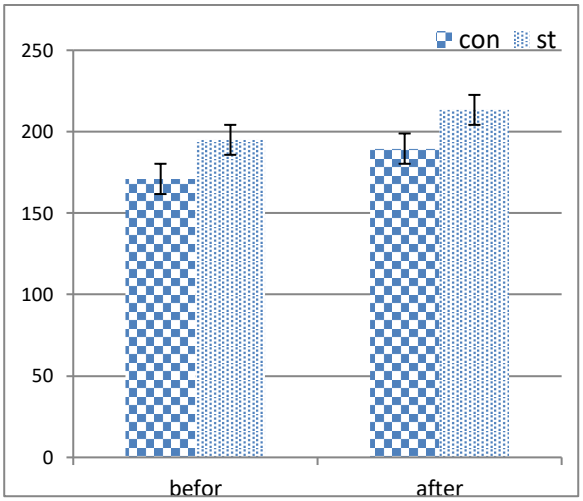

Diagram 1: comparing the rats' weights before and after exposure to styrene in evidence and experimental groups using ANOVA

Liver weights of the rats in the experimental group were 1051±1.18 and the liver weights of the rats in evidence group were 7.62±1.44. The liver weight changes of the rats from evidence and experimental groups was not found statistically significant (P>0.05) (table 2 and diagram 2).

Table 2: comparing the weights of the rats' livers before and after exposure to styrene in experimental and evidence groups using ANOVA test

Variable	Experiment	Evidence	P-value
Liver weight	10.51±1.18	7.62±1.44	P>0.05

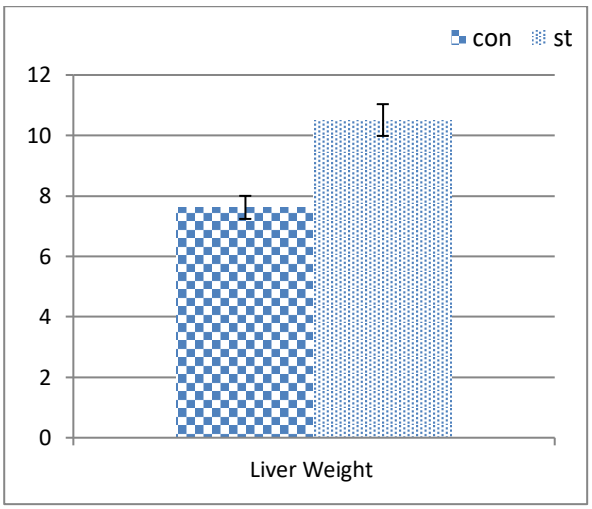

Diagram 2: comparing the rats' liver weights before and after exposure to styrene in evidence and experimental groups using ANOVA test

Degeneration and cell changes were mostly observed in Zone III of the hepatic acinus. Disarray of the hepatic cell ropes was clearly visible in the experimental group and traces of necrosis and apoptosis were found in regions in the periphery of central venule. Mild fatty changes were also evidenced in experimental group. Hydropic degeneration along with cell inflammation, nucleus swelling and eosinophilic cytoplasm reduction was completely visible (figures 1 to 7).

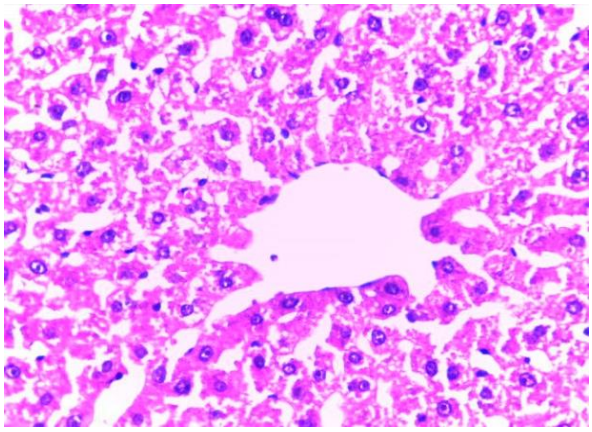

Figure 1: eosinophilic degeneration and vascular dilation in experimental group; eosin and hematoxylin staining, x40 magnification

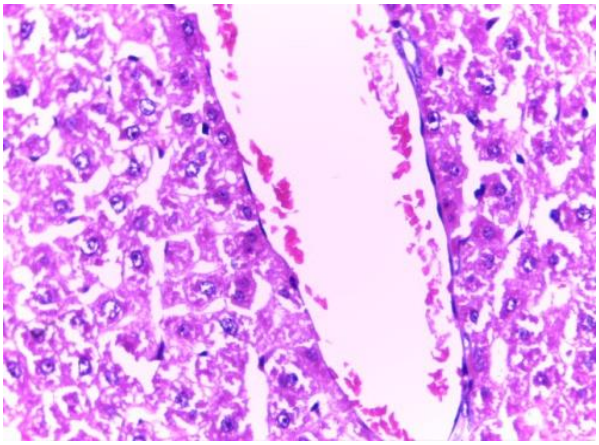

Figure 2: eosinophilic degeneration and vascular dilation in experimental group; eosin and hematoxylin staining, x40 magnification

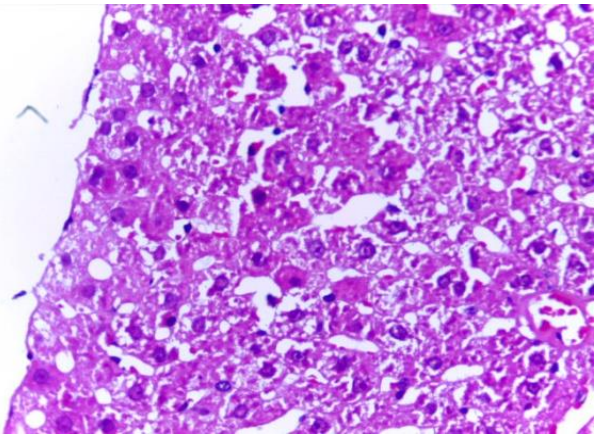

Figure 3: eosinophilic degeneration and vascular dilation in experimental group; eosin and hematoxylin staining, x40 magnification

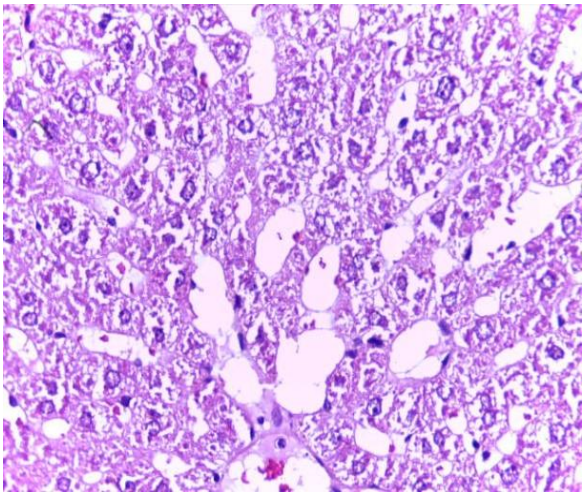

Figure 4: eosinophilic degeneration and vascular dilation in experimental group; eosin and hematoxylin staining, x40 magnification

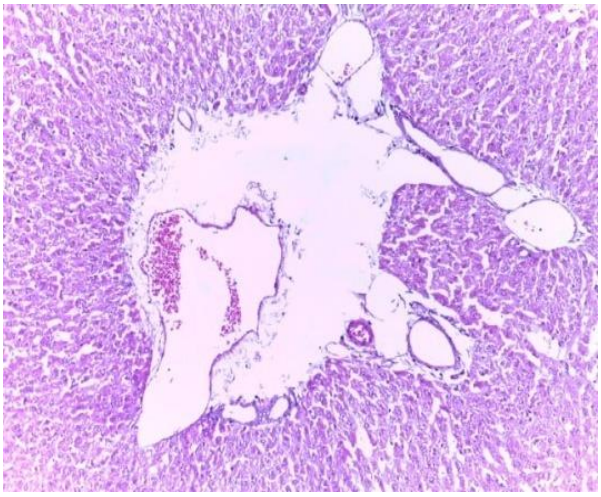

Figure 7: eosinophilic degeneration and vascular dilation in experimental group; eosin and hematoxylin staining, x40 magnification

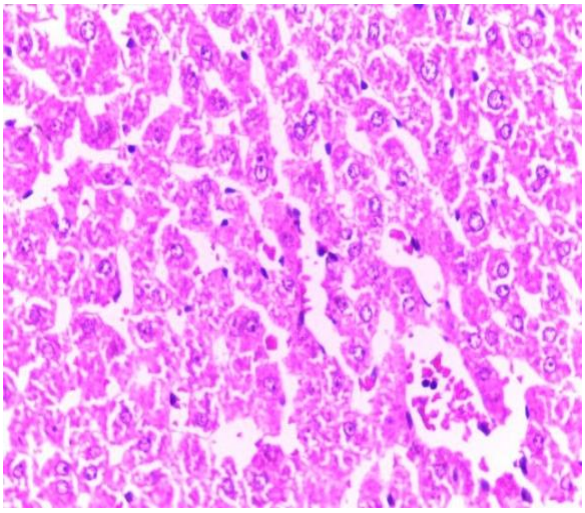

Figure 5: eosinophilic degeneration and vascular dilation in experimental group; eosin and hematoxylin staining, x40 magnification

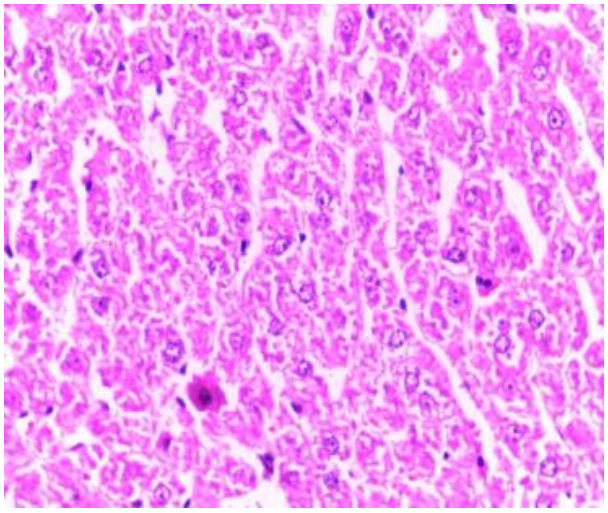

Figure 8: eosinophilic degeneration and vascular dilation in experimental group; eosin and hematoxylin staining, x40 magnification

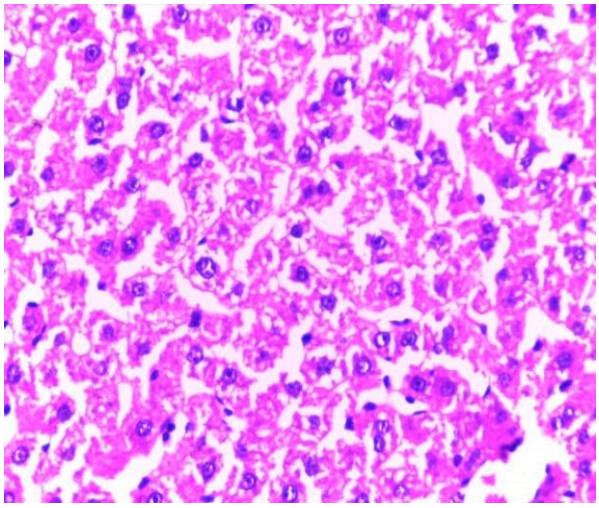

Figure 6: eosinophilic degeneration and vascular dilation in experimental group; eosin and hematoxylin staining, x40 magnification

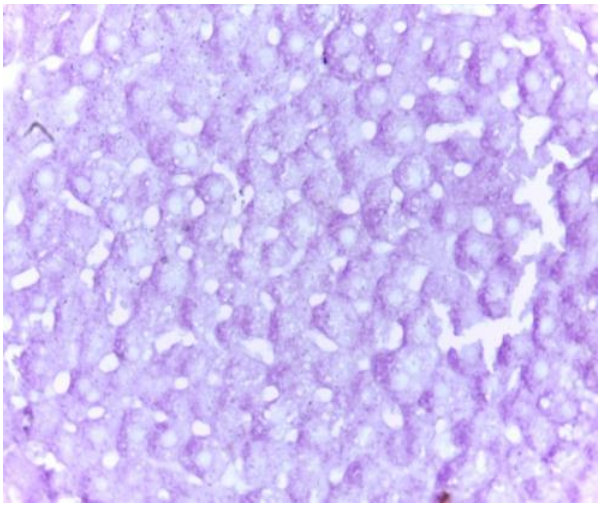

Figure 9: Environmental distribution of glycogen grains in the experimental group, PAS staining, x40 magnification

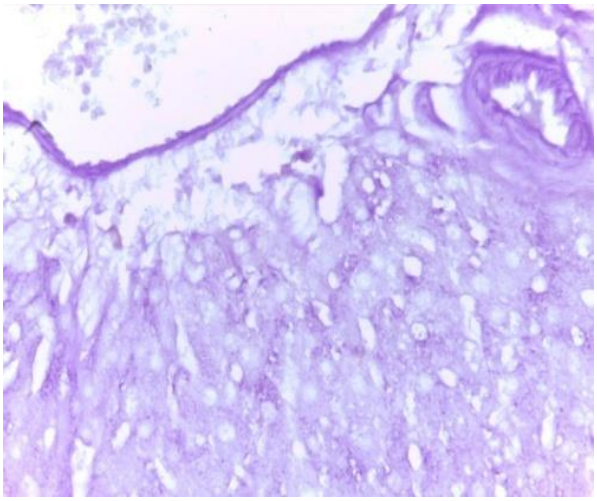

Figure 10: Experimental group specimen; PAS reaction does not exhibit identical patterns in various regions of hepatic lobules, PAS staining, x40 magnification

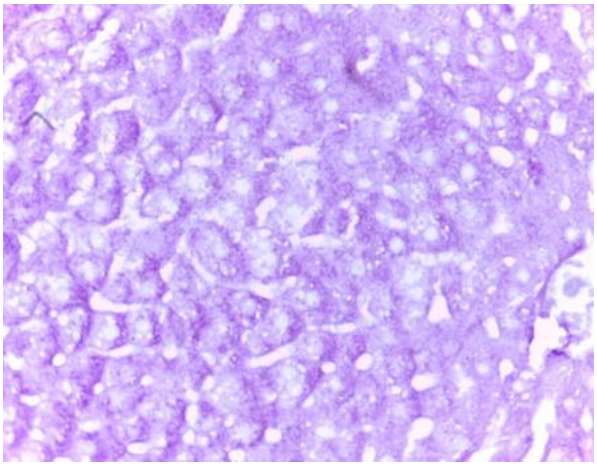

Figure 11: Experimental group specimen; PAS reaction does not exhibit identical patterns in various regions of hepatic lobules, PAS staining, x40 magnification

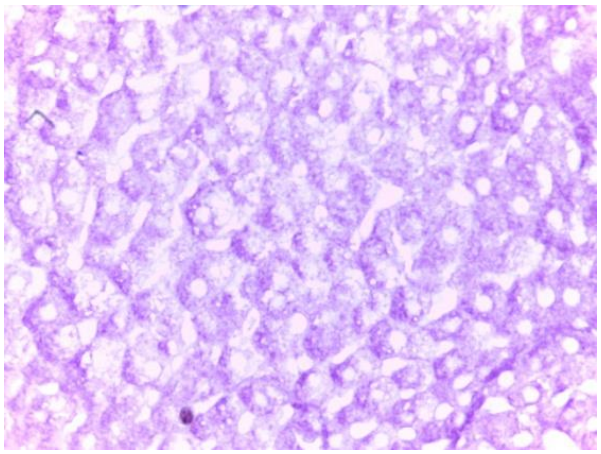

Figure 12: Experimental group specimen; PAS reaction does not exhibit identical patterns in various regions of hepatic lobules, PAS staining, x40 magnification

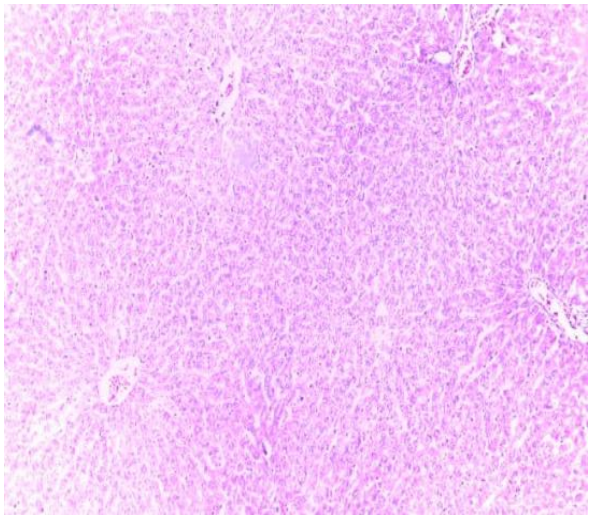

Figure 13: histopathological specimen of the control group; hematoxylin and eosin staining, x10 magnification

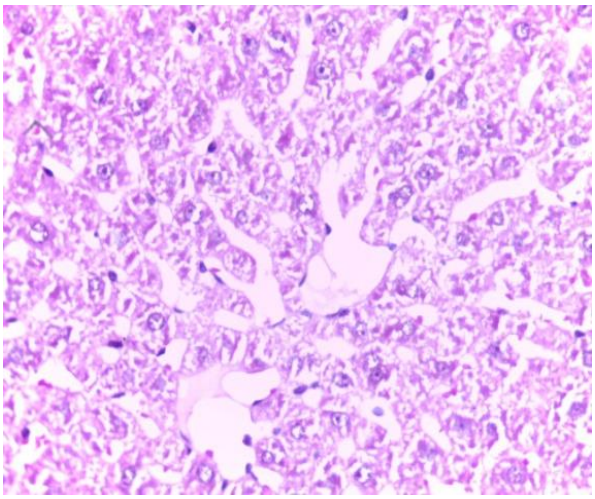

Figure 14: histopathological specimen of the control group; hematoxylin and eosin staining, x40 magnification

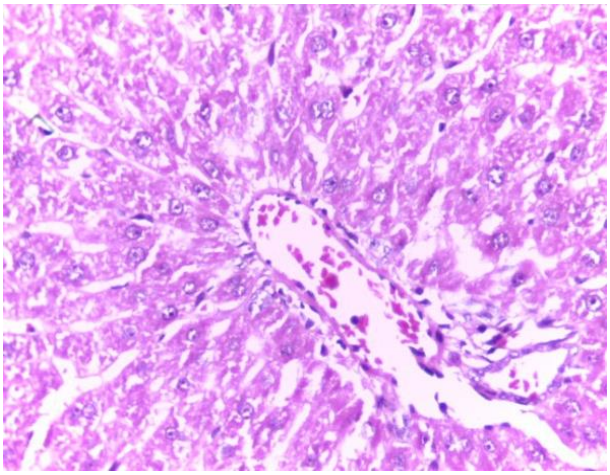

Figure 15: histopathological specimen of the control group; hematoxylin and eosin staining, x40 magnification

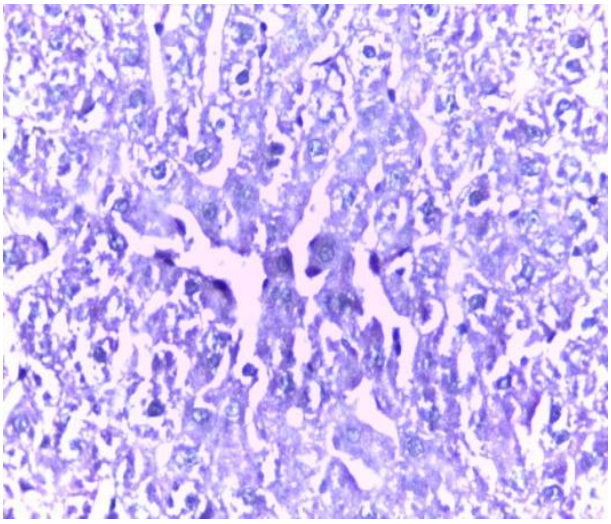

Figure 16: control group specimen, PAS staining, x40 magnification

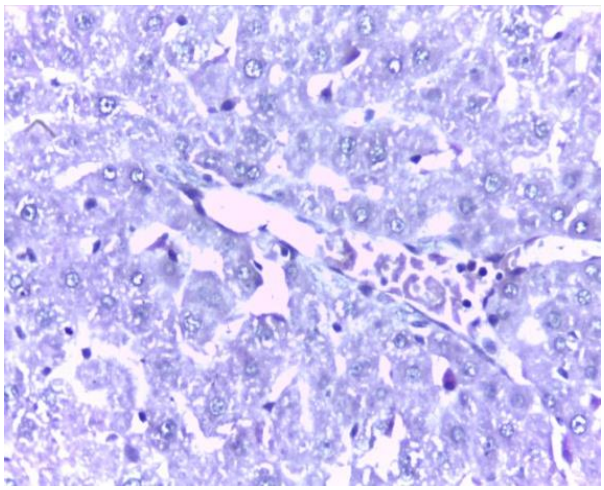

Figure 17: control group specimen, PAS staining, x40 magnification

DISCUSSION AND FINAL CONCLUSION

Upon exposure to styrene, hepatocytes undergo changes that appear in the form of hydropic degeneration along with cell inflammation, nucleus swelling and eosinophilic cytoplasm reduction mostly in Zone III of the hepatic Acinus. In PAS staining, changes were evident in cells in their glycogen environmental distribution patterns. Disarray of hepatic cell ropes and traces of necrosis and apoptosis in regions in the periphery of the central venule were clearly observable. Mild fatty change was observed in experimental group. In the study by Bowen et al in 2009, the increase in liver weight and rats' weight subsequent to styrene vapor inhalation was documented in a one-year research in 1000ppm concentrations. Of course, no significant differences were observed between the groups in terms of the rats' weights. The abovementioned study indicates that the various tissues respond differently to styrene and it is somehow expressive of the different response patterns in various species to styrene [14]. The study by Green et al in 2007 demonstrated that the weight changes between the studied groups subject to styrene exposure are not significant. It was also shown that the female rats are more sensitive than the male ones [15]. The above weight results are in accordance to the results obtained herein. Styrene-induced interspecies differences adds to the importance of histological studies for the determination of the toxic effects of this chemical compound in humans parallel to acquiring a better understanding of the molecular mechanisms [7].

In the study conducted by Aminian et al in 2003, the study results were indicative of the idea that chronic contact with low amounts of styrene in plastic injection industry causes mild cellular damages and hepatic clearance disorders and this is in consistency with what was found in the present study. The hepatic clearance disorder can be a sign of contingent hepatotoxicity risks of these vapors hence the workers in long time exposure to these substances are recommended to regularly perform medical examinations [13]. In 2000, Boogaard et al showed that various species differ in their abilities of detoxifying and excreting metabolites originating from styrene. It seems that mice and rats have detoxification rates equal to 85% and 95%, respectively, for these substances. In the study that was conducted by Kishi et al in 2005 on the gestation effects and gender subject to styrene metabolism in rats' livers, it was determined that pregnancy has negative effects on p450 cytochrome during final stages. Also, it was figured out that pregnancy considerably decreases styrene glycol formation [9]. It was concluded in the studies by Kitamura et al in 2003 that was conducted in line with examining the styrene oligomers' activities after metabolite activation by microsomes of the rats' livers that estrogenic activity of trans-1,2-diphenylcyclobutane (tcb) is induced by 4-hydroxylase metabolite [12].

Simultaneous presence of styrene and the other chemical solvents in work environment and the personal habits of the workers such as smoking and so on and/or the existence of background diseases adds to the problems of doing research in this regard to a large extent [7].

In the extant studies, there is a large deal of diversity of results that can be connected with the individuals' contact levels, use of personal protection instruments and other protective factors and/or be depending on the nature of the common liver examinations that are not generally much accurate. To investigate the changes resulting from exposure to solvents, such other more precise and sensitive tests as bile acids measurement can be employed. The study findings demonstrate that styrene exerts toxic effects on the rats' liver and it causes cell damage and hepatic performance disruptions.

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