HEMATOLOGICAL, BIOCHEMICAL AND HISTOLOGICAL EFFECTS OF PROLONGED EXPOSURE TO n-HEXANE IN ALBINO RATS

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ABSTRACT

n-Hexane is a chemical solvent produced during the cracking and fractional distillation of crude oil and is used widely in industry (paints, varnishes, inks, plastics and rubber). In addition, n-hexane is a major component of benzine and glue which are often sniffed to induce euphoria. The present study aimed to evaluate the effects of prolonged exposure to n-hexane. The experimental animals (albino rats) were dosed with 5 mg/kg n-hexane dissolved in olive oil subcutaneously once daily, 6 days/week for 8 weeks. The hematological findings revealed marked pancytopenia (anaemia, leucopenia, thrombocytopenia) which could be attributed histologically to necrosis and diffuse fibrosis in bone marrow. Hexane-induced neurotoxicity was in the form of mild hemorrhage in brain cortex and could explain the marked decrease in locomotor activity among hexane treated group. The lung tissue revealed moderate hemorrhage with lymphocytic and macrophage infiltration. There were patchy areas of focal hepatocellular necrosis. The cardiac muscle revealed fragmentation that could explain derangement of intracellular ions. There was a significant reduction of myocardial contents of magnesium, potassium and zinc in treated group compared to control group that might have influence upon myocardial excitability. The serum concentration of these elements revealed insignificant difference between treated and control groups. The cardiotoxicity and neurotoxicity of n-hexane could explain mortalities encountered among the treated group throughout the study. The results of the study support the guidelines to minimize occupational exposure to n-hexane and to fight glue, benzine and other solvent abuse.

INTRODUCTION

Solvents are a group of chemicals that have two features: they are liquids and because of their widespread use in commerce, there is a potential for human exposure both during their use and after they have been discarded as chemical wastes (Andrews and Snyder, 1991).

n-Hexane is one of these solvents used for extraction of oils from seeds, and as a component of paints, glues and gasoline. Millions of workers may be exposed to n-

hexane usually by inhalation in occupations such as cabinet finishers, shoes factory, synthetic rubber, petroleum distillation and plastic manufacturing. Small amounts of n-hexane may also leach from hazardous waste sites to ground water sources (Dunnick et al., 1989).

Recently, deliberate inhalation of solvent vapours of a wide variety of substances to induce sensation of euphoria has become a widespread practice. Benzine and glue, which contain hexane in high concentrations, are often sniffed to induce a pleasurable state (Khedun et al., 1992). The sequelae of chronic solvent abuse are brain damage (Lewis et al., 1981), peripheral neuropathy (Anderson et al., 1982), cardiac arrythmias (Reinhardt et al., 1971), optic atrophy (Spencer et al., 1980), hematotoxic and immunotoxic effects (Farris et al., 1997; Robinson et al., 1997).

Taking all these facts into consideration, the present study aimed to evaluate hematological, biochemical and histological effects of prolonged exposure to n-hexane.

MATERIAL AND METHODS

Chemicals :

n-Hexane was obtained from El-Nasr Company for Pharmaceutical Chemicals, Cairo, Egypt. The pure form (exceeding 99%) was colourless liquid with molecular weight 86.18. The weight per ml was about 0.66 gram.

Animals:

Fifty adult male albino rats weighing 150-180 grams were given food and water ad libitum and maintained on 12 hours light/dark cycle. The animals were allowed to stabilize one week prior to the experiment.

The animals were divided into two groups: the control group (20 rats) and the treated group (30 rats). The treated group administered subcutaneous injections of 5 mg/kg n-hexane dissolved in olive oil once daily, 6 days/week for 8 weeks. The control group administered subcutaneous injections of olive oil alone once daily for the same period. The body weight of all animals was recorded before starting and at the end of the experiment. All animals were observed daily for water and food consumption, locomotor activity and colour of fur.

At the end of the experiment, the animals were sacrificed by cut throat and individual blood samples were taken. Each sample was divided into two halves, one half was taken on EDTA tubes for the hematological determination. The other half was left to clot at room temperature, centrifuged at 3000 rpm for 10 minutes and supernated serum was kept frozen at -

20°C for subsequent measurement of serum elements (magnesium, potassium and zinc).

Ten rats from each group were dissected out and their hearts were quickly removed, frozen immediately and stored at -20°C for subsequent measurement of myocardial elements (magnesium, potassium and zinc).

The remaining animals were dissected out, then their brain, heart, lung, liver and bone marrow were taken and fixed in 10% neutral buffered formalin. Bone marrow was decalcified for one hour and all tissue specimens were embedded in paraffin wax. For every specimen, serial sections (5 u) were taken, stained with hematoxylin & eosin for light microscopic examination. Bone marrow was also stained with reticulin stain by silver impregnation.

Hematology:

Red blood cells count (RBCs), hemoglobin concentration (Hb), hematocrit (HT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total white blood cells count (WBCs) and platelets count were determined by automatic hematological analyzer Sysmex E-4000. Blood films were stained with May-Grunwald/Giemsa. Morphological findings and white blood cells differentiation were observed under the microscope.

Measurement of myocardial elements (magnesium, potassium and zinc):

Excised hearts were placed in a 100 ml beaker containing 5 ml of 55% nitric acid. The beaker was placed in a hot boiling bath and digestion allowed to proceed until the heart was completely dissolved. A second beaker containing 10 ml of 60% perchloric acid was placed in a sand bath in a specialised fume cupboard. Anti-bumping granules were added and the perchloric acid allowed to come to boil slowly. The nitric acid solution was added drop by drop to the boiling perchloric acid and the reaction continued until dryness. Distilled water was added to the cooled beaker to a volume of 10 ml and the solution stored at -20°C for subsequent analysis. Tissue magnesium and zinc were analysed by atomic absorption spectrophotometry. Potassium was assayed by flame photometer using caesium chloride as internal standard (Khedun et al., 1992).

Measurement of serum elements (magnesium, potassium and zinc):

Serum magnesium was analysed by atomic absorption spectrophotometry according to Hansen and Freier (1967). Serum potassium was assayed by flame photometry with lithium as internal standard according to Bugyi et al. (1969). Serum zinc was analysed by atomic absorption

spectrophotometry according to the method of Sinaha and Gabriall (1970).

Statistical analysis:

Data were analyzed by Student's t test and percentage according to Armitage (1983).

RESULTS

Survival, body weights and clinical signs :

Five mortalities were encountered in the treated group and recorded after 38 days (one case), 43 days (2 cases) and 52 days (2 cases). There was a decrease in food consumption among treated group relative to control. The final body weight of treated group revealed 12.2% decrease relative to control weight (Table 1). There was a decrease in locomotor activity in the treated group observed from the end of the fourth week and increased gradually in severity till the end of the experiment.

Hematological results:

Pancytopenia was the most common hematological finding in n-hexane treated rats as there was significant decrease in the RBCs, WBCs and platelets count compared to the control (Table 2).

Biochemical results:

Serum levels of magnesium, potassium and zinc in treated group was lower than that in control. The reduction was insignificant as illustrated in table (3).

Myocardial concentrations of these elements are shown in table (4) revealing significant reduction in the hearts of n-hexane treated group compared with control group.

Histopathological results:

The bone marrow, stained with Reticulin stain, revealed necrosis and mild diffuse fibrosis which was encountered in all cases (100%) and illustrated in fig. (1). Vascular changes in the form of endothelial swelling and subendothelial deposition of hyaline-like material in small arteries (Fig. 2) were seen in 12 cases (80%).

The lung tissue of n-hexane treated group, as shown in fig.(3), revealed moderate hemorrhage, congestion, with mononuclear inflammatory cell infiltration, mainly lymphocytes. Some of the macrophages were very large and distended with hemosiderin derived from the ingested red cells. These findings were encountered in 9 cases (66.67%). The heart showed diffuse fragmentation of myocardial fibers (Fig. 4) which was encountered in 80% of cases. There was mild hemorrhage in the brain cortex (100% of cases) shown in fig. (5). The liver revealed patchy areas of focal hepatocellular necrosis (Fig. 6) which was encountered in 80% of cases.

DISCUSSION

The petroleum derivative, n-hexane is an aliphatic hydrocarbon which is used widely as a solvent for many industrial purposes. This chemical solvent is present in high concentrations in glue and benzine which are often sniffed to induce euphoria (Goldfrank et al., 1982; Harada et al., 1999).

Owing to its widespread occupational exposure and abuse by sniffing, it was necessary to evaluate the toxic effects of n-hexane. Being one of the hydrocarbons, the present study aimed to investigate the hematological, biochemical and histological effects of prolonged administration of n-hexane to albino rats.

The results of the study demonstrated that long term exposure to n-hexane had been associated with hematotoxicity and hematopoietic dysfunction and was implicated in the development of pancytopenia (anaemia, leucopenia and thrombocytopenia). This hematotoxicity could be attributed to its direct inhibitory effect on hematopoietic tissue (bone marrow) which revealed necrosis and diffuse fibrosis on microscopic examination. These findings support the fact that aplastic anaemia, leucopenia and thrombocytopenia are the main hematotoxic effects associated with hydrocarbons toxicity (Goldfrank et al., 1982). The bone marrow vascular changes observed in the present study, could be attributed to gamma globulin deposition by the surrounding plasma cells, alternatively, the result of immune complex deposition. The bone marrow fibrosis, in the form of increased reticulin, can be explained by the increased proliferation and/or necrosis of histiocytes (Farris et al., 1997; Robinson et al., 1997).

The hepatotoxicity manifested by diffuse hepatocellular necrosis and pulmonary toxicity in the form of moderate hemorrhage and lymphocytic infiltration could be attributed to the direct action of n-hexane on these target organs.

The mild hemorrhage in brain cortex of n-hexane treated rats proved nervous tissue damage and could explain the marked decrease in their locomotor activity. These findings support the results of Wuldron (1981) who observed that n-hexane caused degeneration of central and peripheral nervous tissues in rats. Also, Cavender et al. (1984) reported nerve damage after n-hexane exposure at 10000 ppm for 13 weeks in rats. The n-hexane induced neurotoxicity, as mentioned by Krasavage et al. (1980) can be attributed to its major metabolite 2, 5-hexanedione which is many times more potent than the parent chemical.

The cardiac lesion was in the form of myocardial fragmentation which indicat-

ed the hypercontraction of the myocardium. The cardiac lesions have a role in the arrhythmogenesis and cardiac failure as stated by Cunningham et al. (1987) who reported primary ventricular fibrillation and myocardial infarction in a patient following a prolonged period of glue sniffing. Also, Maharaj et al. (1993) reported on dissociation, reduction of diameter of the myofilament, oedema and degeneration of the mitochondria in hearts of n-hexane treated rats.

In the present study, n-hexane caused derangement of the intracellular ions within the myocardium resulting in myocardial electrolyte deficiency. These electrolytes, according to Khedun et al. (1992), may have influence upon myocardial excitability and consequently may play a role in n-hexane induced arrhythmia. Magnesium has an important role in cardiac rhythmicity and loss of cellular magnesium may result in the reduction of Mg^{+2}-ATPase affecting the (Na^+, K^+) pump thereby altering membrane potential and consequently cardiac rhythm. Intracellular potassium deficiency is associated with a decrease in resting membrane potential which increases myocardial excitability. Zinc frequently acts as an essential cofactor to many enzymatic reactions.

The histological findings could explain mortalities in this study which might be combinations of cardiac, respiratory and central nervous system disorders. These explanations could prove theories that had been postulated as to how chronic solvent exposure and abuse might cause death. They include respiratory depression, cardiac depression, inhalation of gastric contents while unconscious, suffocation and hypoxia. However, in many cases the collapse is sudden suggesting a lethal cardiac arrhythmia (Streicher et al., 1981; Harada et al., 1999). It is thought that solvent abuse causes sudden death directly or by sensitizing the heart to the arrhythmogenic action of adrenaline (Khedun et al., 1992).

As the metabolism of hydrocarbons in the rat has been shown to closely mimic that of humans (Seaton et al., 1995), the results of this study support the guidelines to minimize human exposure to n-hexane.

The current Occupational Safety and Health Administration (OSHA) standard for n-hexane is 500 ppm (1800 mg/m3) averaged over an 8 hour work shift. The American Conference of Government Industrial Hygienists recommended that the permissible exposure limit for n-hexane is 50 ppm (180 mg/m3) averaged over a work shift of up to 10 hours/day, 4 hours/period (Dunnick et al., 1989).

Thousands of workers may be exposed to n-hexane usually by inhalation in occupation for prolonged time. Also, benzine

and glue, products consisting principally of n-hexane, are bought freely by a large number of street children and sniffed to induce euphoria. In addition, the ground water is a considerable source for water consumption which may be contaminated with hexane by leakage from hazardous waste sites (Dunnick et al., 1989; Khedun et al. 1992). Therefore, periodic measurement of environmental concentration of n-hexane in air and drinking water inside and near industrial areas is recommended. Also, periodic clinical examination and blood cells counting of workers at high risk for n-hexane exposure is recommended to detect any signs of toxicity as early as possible to get better prognosis.

It must be considered that the major drawbacks of most studies on the toxicology of solvents is that actual occupational and environmental exposure is not to the pure compounds used in toxicological research but to commercial mixtures of solvents. Therefore, further researches are recommended to develop strategies for studying the toxicology of solvent mixtures.

Table (1) : Body weight of control and n-hexane treated groups.

Group	Body weight (gram)		Final wt. % of control
	Initial Wt. Mean ±SD	Final Wt. Mean ±SD	
Control (n= 20)	164±12	197±8	100%
Treated (n= 25)	161±9	173±6*	87.8%

* Significant ($P < 0.05$).

Table (2) : Hematological picture in control and n-hexane treated groups.

Hematological parameter	Control group (n= 20) Mean ± SD	Treated group (n= 25) Mean ± SD
RBCs ($X10^6$ /ul)	9.21±0.13	6.34±0.15*
Hb (g/dl)	16.3±0.6	13.2±0.3*
Ht (%)	47.2±1.1	39.7±0.5*
MCV (fl)	50.8±1.0	42.4±1.4*
MCH (pg)	18.1±0.3	15.3±0.5*
MCHC (g/dl)	35.4±0.5	31.5±0.6*
WBCs ($X10^3$ /ul)	6.2±0.3	3.7±0.5*
Neutrophils (%)	17.5±5.7	14.8±4.6
Lymphocytes (%)	80.7±5.4	83.4±4.2
Monocytes (%)	0.8±0.6	0.7±0.5
Eosinophils (%)	1.0±0.9	1.1±0.8
Platelets ($X10^3$ /ul)	640±44	453±36*

* Significant ($P < 0.05$).

Table (3): Serum concentrations of magnesium, potassium and zinc in control and n-hexan treated rats.

Group	Magnesium (mg/dl)	Potassium (mEq./L)	Zinc (ug/dl)
	Mean ± SD	Mean ± SD	Mean ± SD
Control (n= 20)	1.84±0.32	4.12±0.42	110.86±5.48
Treated (n= 25)	1.68±0.44	3.84±0.68	106.64±6.18

Insignificant ($P > 0.05$).

Table (4) : Concentrations of magnesium, potassium and zinc in the heart of control and n-hexane treated rats.

Group	Magnesium (ug/g)	Potassium (ug/g)	Zinc (ug/g)
	Mean ± SD	Mean ± SD	Mean ± SD
Control (n= 10)	204±16	2946±212	32.6±6.2
Treated (n= 10)	162±24*	2568±146*	20.8±4.6*

Significant ($P < 0.05$).

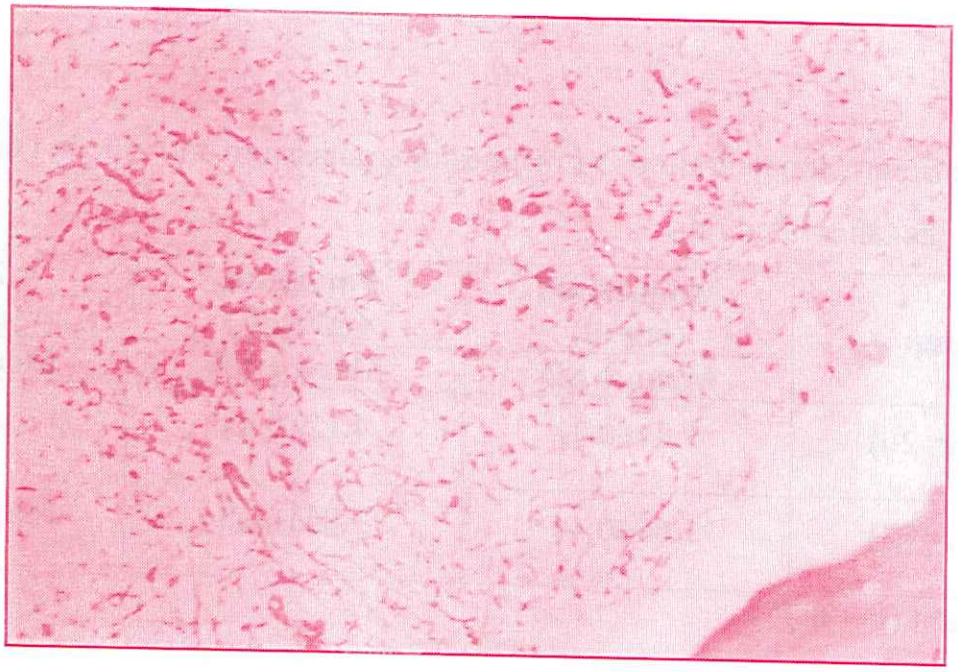

Fig. (1) : Section in the bone marrow exhibiting diffuse fibrosis. **(Reticulin stain X 250)**

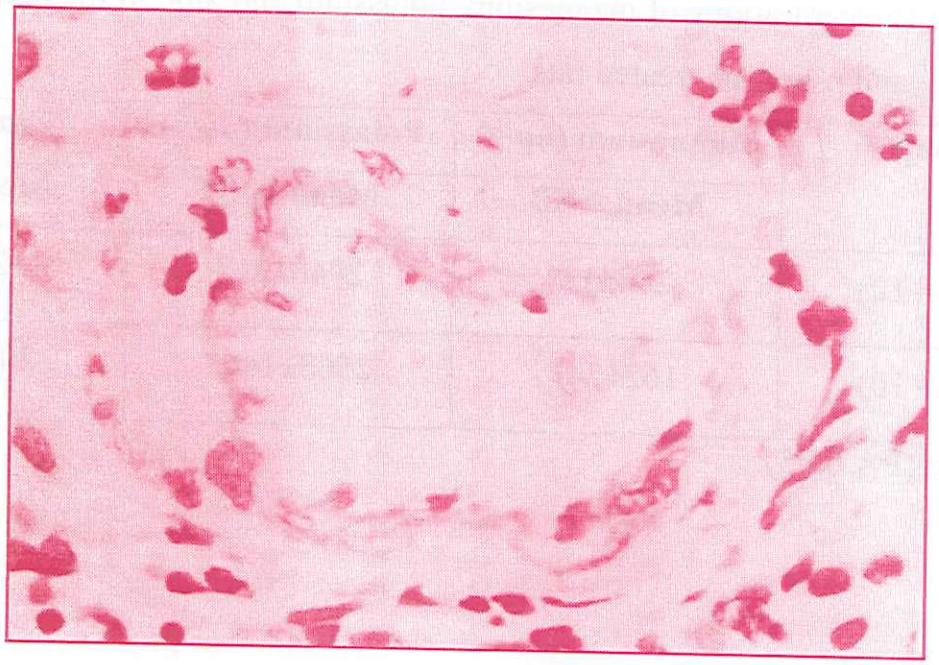

Fig. (2) : Section in the bone marrow showing endothelial cell swelling and subendothelial deposition of hyaline like material. **(H & E X 400)**

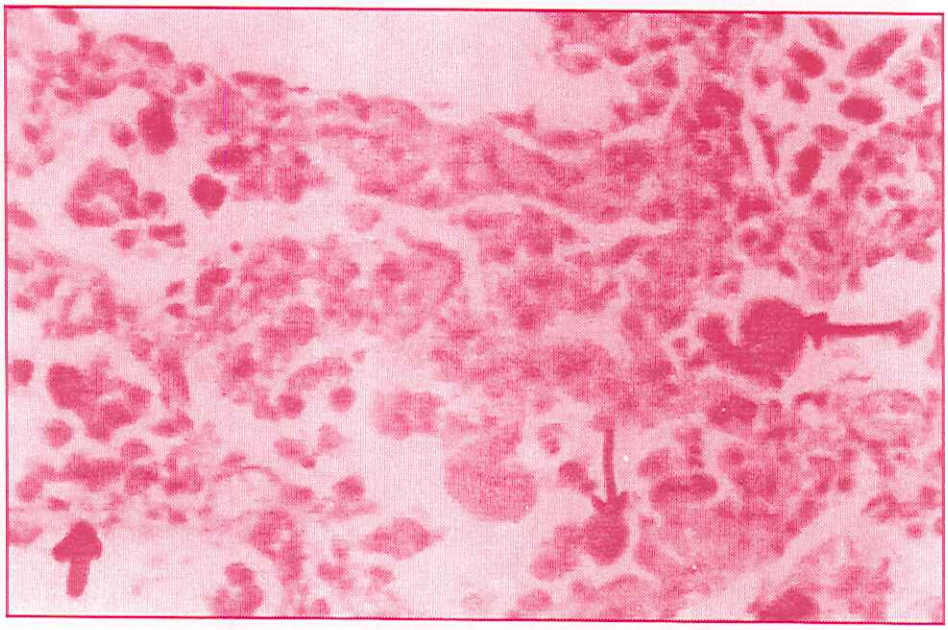

Fig. (3) : Section in the lung tissue showing moderate hemorrhage with mononuclear inflammatory cell infiltration, mainly lymphocytes. Some macrophages are very large and distended with hemosiderin (brown pigment) derived from ingested red cells. **(H & E. X 200)**

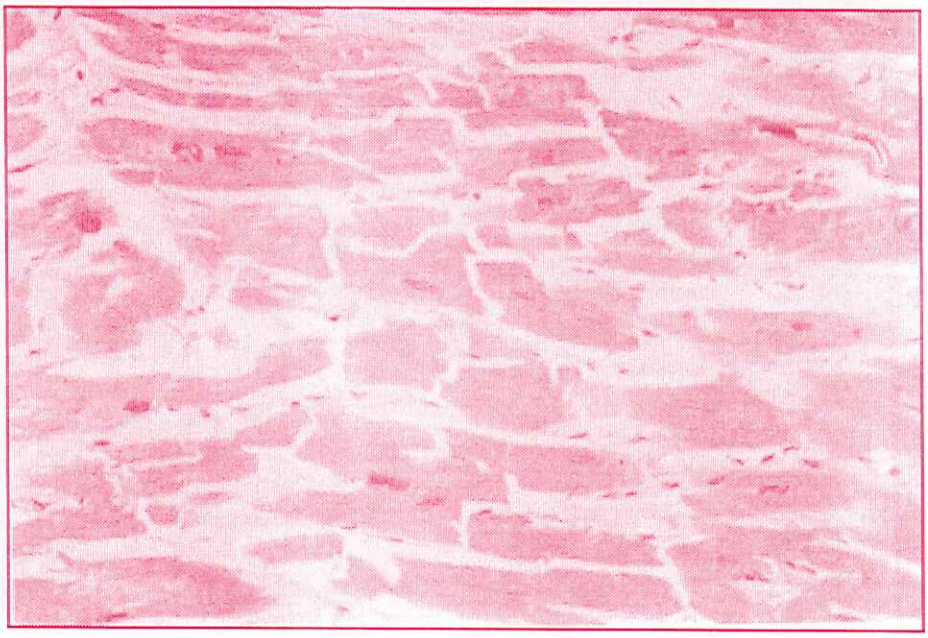

Fig. (4): Section in the cardiac muscle showing fragmentation of cardiac muscle fibers. **(H & E X 400)**

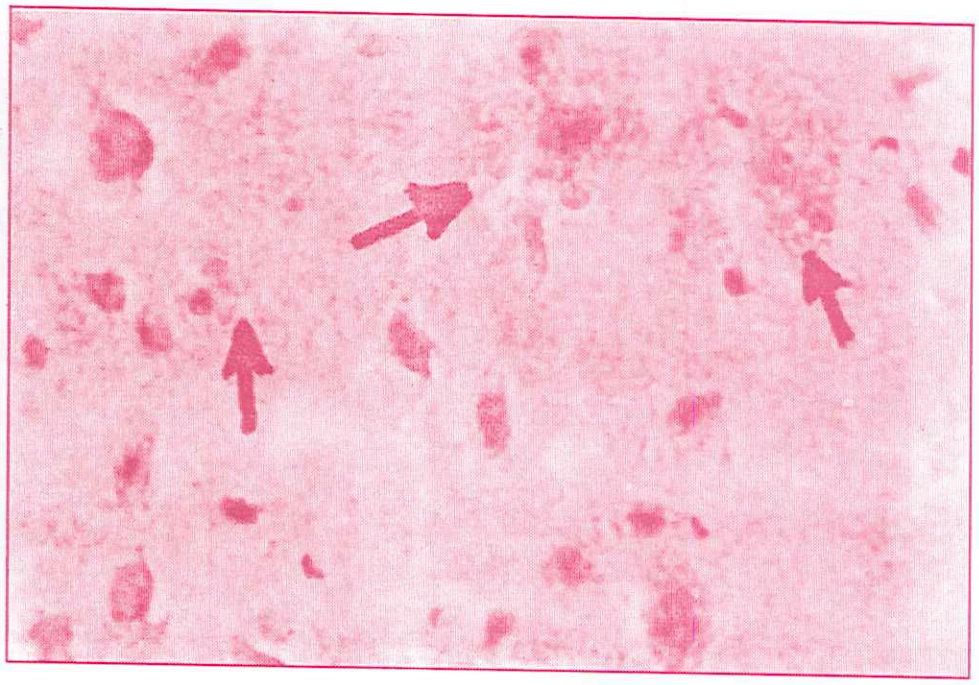

Fig. (5) : Section in the brain tissue exhibiting mild hemorrhage. **(H & E X 400)**

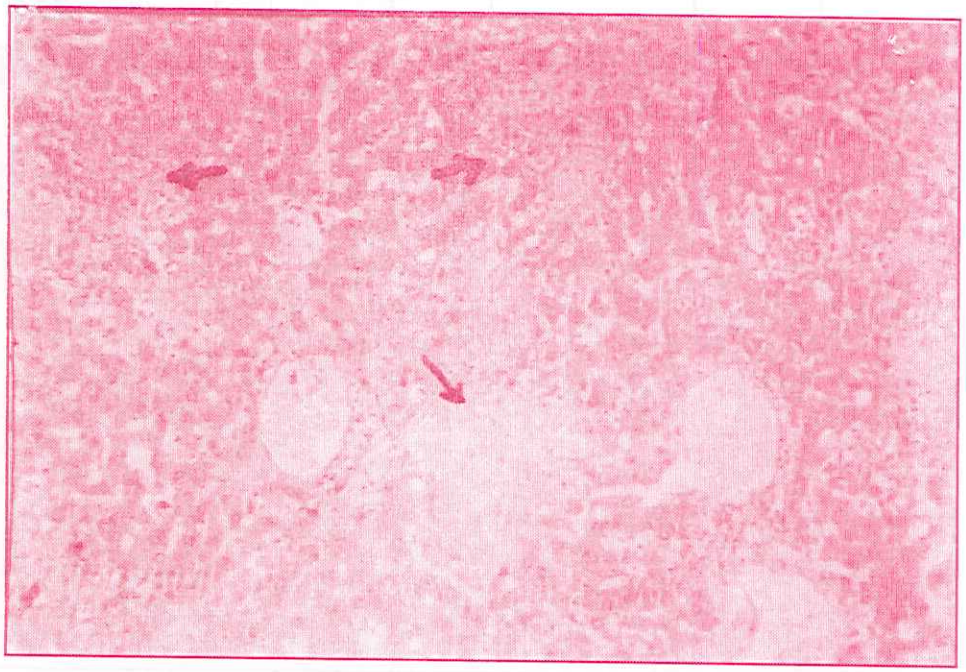

Fig. (6) : Section in the liver showing patchy areas of focal hepatocellular necrosis . **(H & E X 120)**

REFERENCES

Anderson, Hr; Dick, B.; MacNair, R. S.; Pulmer, J. C. and Ramsey, J. D. (1982) : "An investigation of 140 deaths associated with volatile substance abuse in U.K." Hum. Toxicol., (1): 201-221.

Andrews, L. S. and Snyder, R. (1991) : Toxic effects of solvents and Vapors. In : Casarett and Doull's Toxicology : The Basic Science of Poisons. Amdur, MO; Doull, J.; Klaassen, CD (Eds). 4th ed., Pergamon Press Inc., P.P. 681-722.

Armitage, P. (1983) : Statistical Methods in Medical Research. Blackwell Scientific Publication, Oxford, London.

Bugyi, H. I.; Magnier, E.; Joseph, W. and Frank, G. (1969) : "A method for measurement of sodium and potassium in erythrocytes and whole blood". Clin. Chem., (15): 712.

Cavender, F. I; Casey, H. W.; Salem, S. H.; Graham, D. G.; Swenberg, J. A. and Gralla, E. J. (1984) : "A 13-week Vapor inhalation study of n-hexane in rats with emphasis on neurotoxic effects". Fundam. Appl. Toxicol., (4): 191.

Cunningham, S. R.; Dalzell, G. W. N.; McGirr, P. and Khan, M. M. (1987) : "Myocardial infarction and primary ventricular fibrillation after glue sniffing". Br. Med. J., (294): 739-740.

Dunnick, J. K.; Graham, D. G.; Yang, R. S. H.; Haber, S. B. and Brown, H. R. (1989) : "Thirteen week toxicity study of n-hexane in B6C3F1 mice after inhalation exposure". Toxicology, (57): 163-172.

Farris, G. M.; Robinson, S. N.; Wong, B. A.; Wong, V. A.; Hahn, W. P. and Shah, R. (1997): "Effects of benzene on splenic, thymic and femoral lymphocytes in mice". Toxicology, 118: 137-148.

Goldfrank, L. R.; Kirstein, R. and Bresnitz, E. A. (1982) : Hydrocarbons. In : Toxicologic Emergencies: A Comperhensive Handbook in Problem Solving. Goldfrank, LR, Flomenbaun, NE.; Lewin, NA.; Weisman, RS. (Eds.), Appleton-Century Crofts, New York. P.P. 337-345.

Hansen, J. L. and Freier, E. F. (1967) : "The measurement of serum magnesium by atomic absorption spectrophotometry". Am. J. Med. Technol., 33: 158.

Harada, K.; Ichiyama, T.; Ikeda, H. and Yoshida, K. (1999) : "A fatal case of oral ingestion of benzine". The American Journal of Forensic Medicine and Pathology, 20(1): 84-89.

Khedun, S. M.; Mahraj, B.; Leary, W. P. and Lockett, C. J. (1992) : "The effect of hexane on the ventricular fibrillation

threshold of the isolated perfused rat heart". Toxicology, 71:145-150.

Krasavage, W. J.; O'Dunoghue, J. L.; Divincenzo, G. D. and Terhaar, C. J. (1980) : "The relative neurotoxicity of methyl n-butyl ketone, n-hexane and their metabolites". Toxicol. Appl. Pharmacol., 52: 433-441.

Lewis, J. D.; Mortiz, D. and Melis, L. P. (1981) : "Long term toluene abuse". Am. J. Psychiatry, 138: 368-370.

Mahraj, B.; Khedun, S. M.; Gregory, M. A. and Naicker, T. (1993) : "The effects of hexane on rat myocardium: a morphometric and morphological study". Int. J. Exp. Pathol., 74: 145-150.

Reinhardt, C. F.; Azar, A.; Maxfield, M. E.; Smith, Jr. P. E. and Mullin, I. S. (1971) : "Cardiac arrythmias and aerosol sniffing." Arch. Environ. Health, 22: 265-279.

Robinson, S. N.; Shah, R.; Wong, B. A.; Wong, V. A. and Farris, G. M. (1997) : "Immunotoxicological effects of benzene inhalation in male sprague-dawley rats". Toxicology, 119: 227-237.

Seaton, M. J.; Schlosser, P. M. and Medinsky, M. A. (1995) : "In vitro conjugation of benzene metabolites by human liver: Potential influence of interindividual variability on benzene toxicity". Carcinogenesis, 16: 1519-1527.

Sinaha, S. N. and Gabriall, E. R. (1970) : "Serum coppper and zinc levels among various pathological conditions". J. Clin. Pathol., 54: 570-574.

Spencer, P. S.; Schaumburg, H. H.; Sabri, M. I. and Veronesi, V. (1980) : "The enlarging view of hexacarbon neurotoxicity". CRC Crit. Rev. Toxicol., 7: 279-345.

Streicher, H. Z.; Gabow, P. A.; Moss, A. H.; Kono, D. and Kaehny, W. D. (1981) : "Syndromes of toluene sniffing in adults". Ann. Intern. Med.,14: 758-762.

Wuldron, H. A. (1981) : "Effects of organic solvents". Br. J. Hosp. Med., 26: 645.

التأثيرات الدموية والبيوكيميائية والهستولوجية الناتجة عن التعرض إلى ن-هكسان لفترة طويلة فى جرذان التجارب البيضاء

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يعتبر ن-هكسان أحد المذيبات الكيميائية الناتجة من التقطير الجزئى لزيت البترول الخام، ويستخدم فى صناعة الكثير من أنواع الطلاء والورنيش والأحبار والمطاط والبلاستيك، كذلك يعتبر ن-هكسان أحد المكونات الرئيسية لبعض المذيبات مثل البنزين والكلة والتى يعتاد الكثير من مدمنى الطبقات الفقيرة وخاصة أطفال الشوارع استنشاقها.

ويهدف البحث إلى دراسة التأثير الدموى والبيوكيميائى والهستولوچى الناتج عن التعرض إلى ن-هكسان لفترة طويلة فى جرذان التجارب البيضاء، وقد تم إعطاء الجرذان جرعات من ن-هكسان مذاباً فى زيت الزيتون بجرعة ٥ مجم / كجم تحت الجلد يومياً ستة أيام فى الإسبوع لمدة ثمانية أسابيع.

وقد أوضحت نتائج فحص صورة الدم الكامل فى الجرذان التى تم حقنها وجود نقص ذو دلالة إحصائية واضحة فى عدد خلايا الدم الحمراء والبيضاء وكذلك الصفائح الدموية نتيجة ضمور شديد وتليف فى النخاع العظمى. وأظهر الفحص الميكروسكوبى للمخ نزيف فى قشرة المخ والذى يفسر نقص النشاط العصبى الحركى فى الجرذان وأظهر الفحص الميكروسكوبى لعضلة القلب تلف، وأظهر فحص الكبد نخر، كذلك أظهر فحص الرئة نزيف بدرجة متوسطة داخل الحويصلات الهوائية مع إرتشاح الخلايا الليمفاوية. كذلك أظهرت الدراسة وجود نقص ذو دلالة إحصائية واضحة فى مستوى الماغنسيوم والبوتاسيوم والزنك فى عضلة القلب فى الجرذان التى تم حقنها بالهكسان مقارنة بالمجموعة الضابطة بينما أظهر مستوى هذه العناصر فى مصل الدم عدم وجود إختلاف ذو دلالة إحصائية واضحة مقارنة بالمجموعة الضابطة، وأرجعت الدراسة حدوث الوفيات بين الجرذان التى تم حقنها بالهكسان لتأثيره السمى على الجهاز العصبى والقلب.

تؤكد نتائج الدراسة على أهمية التقليل من التعرض إلى ن-هكسان إلى أدنى مستوى خاصة بين العمال فى الصناعات السابق ذكرها وعلى ضرورة محاربة ظاهرة إدمان استنشاق المذيبات خاصة البنزين والكلة والتى تحتوى على نسبة عالية من ن-هكسان نظراً لتأثيره المدمر على الجهاز العصبى والدموى والقلب.