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STUDIES ON THE TOXICITY OF LINDANE IN ALBINO RAT: HISTOPATHOLOGICAL EFFECTS IN LIVER

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Abstract: An organochlorine insecticide, lindane (gamma-HCH) was administered to three groups of Sprague Dawley rats, @ 30 mg/kg body wt. once, 18 mg/kg body wt./day and 9 mg/kg body wt./day, for 48 hours, 15 days and 18 months, respectively. Following stipulated durations, animals were dissected, their livers were removed, fixed quickly and processed further by routine histological techniques. Liver sections, 6-8 μm thick were cut, stained with haematoxylin and eosin technique and examined under microscope for morphometric and pathological changes. Lindane treatment produced significant increase (10-59%) in hepatic cell size in all experiments. The nuclear and nucleolar size also showed similar increase with 26% and 73% maximum change. The number of cells/microscopic field decreased by 18%, 22% and 26% in 48 hour, 15 days and 18 month experiments. No significant change was found in number of nuclei/cell and number of nucleoli/nucleus. The liver sections revealed moderate to high cellular pathology in different treatments. Cellular and nuclear hypertrophy was evident in almost all treatments. The clear areas in the cytoplasm showed proportionate increase with increase in duration of insecticide treatment. Increased sinusoidal spaces, condensed/hyperchromatic and distorted nuclei, fatty changes, increase in kupffer cells and clear areas around the nuclei were the other prominent changes.

Key words: Chlorinated hydrocarbons, gamma-benzenehexachloride (gamma-BHC), gamma-hexachlorocyclohexane (gamma-HCH), insecticides, liver histology, liver pathology, morphometric changes, organochlorines, pesticides, mammals, vertebrates.

INTRODUCTION

Pesticides play a very significant role in boosting agriculture production. In present times, practically we can not feed the world by excluding pesticides from the agriculture sector.

Lindane (gamma-HCH) is one of such pesticides, belonging to organochlorine group, which has been extensively used in the near past for controling crop pests in agriculture and vector-borne diseases in public health and veterinary sectors. This chlorinated insecticide has shown great cummulative potential due to hydrophobic nature of its benzene ring, the characteristic which enable this insecticide to bind with lipoprotein membrane system in the cell. The principle binding force, of course, is hydrophobic interaction between the insecticide molecules and cell membranes. Due to this property, the residues of these chlorinated insecticides are being reported from soil, water, animals and plant tissues throughout the world (Lopez Carrillo *et al.*, 1996; Dua *et al.*, 1996). In living systems, the residues and their metabolites frequently induce

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serious consequences such as reduction in weight, liver injury, (Ali and Shakoori, 1990, 1996; Mari *et al.*, 1994; Ito *et al.*, 1996) convulsions and tremors followed by death (Nagata and Narahashi, 1995; Nagata *et al.*, 1996; Blaszczak and Turski, 1998).

Toxicity of sublethal doses of lindane and other related organochlorine insecticides in animals has already been reported from different laboratories (Blaszczak and Turski, 1998). According to one study, lindane significntly reduced the T3 and T4 concentrations while TSH level was increased (Akhtar *et al.*, 1996). Previous studies have also shown that chlorinated insecticides are liver tumor promoters in rat and other animals (Davis *et al.*, 1993; Leiss and Savitz, 1995; Lopez Carrillo *et al.*, 1996; Ito *et al.*, 1996; Rougnt *et al.*, 1998).

In the present study histopathological effects of lindane are being reported in the liver of rat because liver is invovlved in the further processing and transformation of insecticides and considered as the main target of foreign chemical invasion.

MATERIALS AND METHODS

Experimental animals and their maintenance

Three groups of albino rats (Sprague Dawley strain) were fed on three different dose levels of lindane, orally, alongwith the feed for the total duration of 48 hours, 15 days (strong dose experiments) and 18 months (week dose experiment). The animals were kept in iron cages under optimum conditions in small groups. The feed and water was provided to rats ad libitum. The rat feed was formulated as mentioned in Ali and Shakoori (1988).

Toxicant used and its administration

A polychlorinated organic compound, lindane [gamma- hexachloro-cyclohexane (HCH), gamma-benzene hexachloride (BHC)] was obtained as 26% powder from Jafar Brothers (Pvt.) Ltd. Shadman, Lahore. The insecticide was administered to rats @ 30 mg (=0.33 LD_{50}), 18 mg (=0.20 LD_{50}) and 9 mg (0.10 LD_{50})/kg body wt./day for three different durations *i.e.*, 48 hours, 15 days and 18 months, respectively.

The insecticide-mixed diet for first strong dose (30 mg) experiment was prepared by adding 3.84 g of lindane (26%) per kg of rat feed. In second strong dose (18 mg) experiment 2.31 g of lindane/kg was mixed with rat feed while weak dose feed was prepared by the addition of 1.15 g of lindane/kg of rat feed thoroughly with the help of suitable amount of water to prepare semi-solid cakes.

Experimental procedure

Three to five animals each from treated and control groups were dissected to collect the liver samples. Small pieces of liver were quickly fixed in Bouin's fixative and processed further for routine paraffin embedding technique. Sections of suitable thickness (6-8 μm) were prepared and stained with haematoxylin and eosin counter staining technique. The histological sections were examined under microscope for morphometric changes and for induction of any other pathology by lindane.

Ocular micrometer, pre-calibrated with stage micrometer, was used for all measurements. For counting cells their nuclei and nucleoli 500X magnification while for measuring size 1250X magnification was used.

RESULTS

Tables I-III and Figures 1-3 show the effect of feeding gamma-HCH mixed diet, @ 30 mg, 18 mg and 9 mg/kg body wt./day for the total period of 48 hours, 15 days and 18 months, respectively on various morphometric parameters of rat liver. As it is obvious from the data that hepatic cells, their nuclei and nucleoli were hypertrophied after insecticide treatment. The hepatic cell size increased 14% and 39% after 24 and 48 hours of toxicant feeding. The nuclear size increased by 26% and 18%, while nucleolar size increased 42% and 35% during the same fedding duration, respectively. The number of nuclei/cell and number of nucleoli/nucleus did not show any alteration after insecticide treatment @ 30mg/kg body wt./day for 48 hours (Table I, Fig.1).

During 15 day lindane treatment the hepatic cell size increased gradually and showed 30% increase after 15 days of insecticide feeding. The nucleolar size increased 20% during the same period and showed 52% increase during first week of insecticide administration. The size of the nucleoli no longer showed any significant deviation from control during later part of the experiment. The number of nuclei/cell and the number of nucleoli/nucleus did not show any change, although the number of cells/microscopic field decreased by 22% (n=90) after 15 days of gamma-HCH feeding (Table II, Fig.2).

Table I: Effect of feeding gamma-HCH mixed diet (30 mg/kg body weight/day) for 48 hours on the various histological parameters of rat liver.

Parameters	Control	gamma-HCH feeding	
		24 hours	48 hours
No. of cells/ field (n=9)	276.54[a] ±9.62	247.92 ±11.39	226.13* ±14.45
No. of nuclei/ cell (n=90)	1.12 ±0.33	1.19 ±0.05	1.26 ±0.15
No. of nucleoli/ nucleus (n=90)	1.52 ±0.17	1.77 ±0.09	1.63 ±0.13
Size of cell (μm^2; n=90)	269.27 ±8.49	306.91** ±4.92	373.95** ±9.09
Size of nucleus (μm^2; n=90)	37.71 ±0.92	47.39*** ±1.18	44.45** ±1.33
Size of nucleolus (μm^2; n=90)	2.81 ±0.18	3.98* ±0.30	3.80** ±0.21

[a]Mean ± SEM. Student's 't' test; *P<0.05; **P<0.01; ***P<0.001

Following 18 months of uninterrupted gamma-HCH feeding @ 9 mg/kg body weight/day, the hepatic cells, their nuclei and nucleoli registered a significant increase. The average hepatic cell size increased 34, 28 and 59%, respectively, after 6, 12 and 18 months toxicant feeding, while the nucleus showed 20, 13 and 14% and nucleolus 73, 42 and 37% increase during the same period. The number of nuclei/cell and the number of nucleoli/nucleus did not show any significant deviation. The number of cells/microscopic field were decreased due to the hypertrophy of the hepatic cells (Table III, Fig.3).

Figures 8-29 show the histological structure of rat liver following lindane feeding at various dose levels for 48 hours, 15 days and 18 months. During 48 hours insecticide feeding, the hepatolobular structure was maintained, though very prominently hypertrophied hepatic cells with vesiculated nuclei, prominent nucleoli and granulated cytoplasm was observed (Figs. 10 and 12) when compared with the normal section (Fig.7). Slight cellular disorganization was evident from irregular arrangement of cells in addition to distorted and condensed nuclei (Figs. 8 and 11).

Table II: Effect of feeding gamma-HCH mixed diet (18 mg/kg body weight/day) for 15 days on the various histological parameters of rat liver.

Parameters	Control	gamma-HCH feeding				
		3 days	6 days	9 days	12 days	15 days
No. of cells/ field (n=9)	274.64[a] ±12.47	253.32 ±8.40	231.17* ±9.63	223.54* ±10.19	220.40* ±11.21	213.15** ±10.44
No. of nuclei/ cell (n=90)	1.08 ±0.04	1.17 ±0.04	1.34 ±0.20	1.16 ±0.04	1.10 ±0.03	1.14 ±0.04
No. of nucleoli/ nucleus (n=90)	1.49 ±0.11	1.54 ±0.09	1.71 ±0.26	1.51 ±0.08	1.62 ±0.10	1.65 ±0.10
Size of cell (μm^2; n=90)	289.23 ±8.81	317.01* ±5.61	330.32** ±5.03	345.42** ±6.45	335.95** ±7.84	375.11*** ±10.94
Size of nucleus (μm^2; n=90)	39.45 ±1.18	45.79* ±1.49	47.93** ±1.44	47.90** ±1.42	46.44** ±1.20	47.52** ±1.28
Size of nucleolus (μm^2; n=90)	2.57 ±0.30	3.68* ±0.22	3.90* ±0.25	3.44* ±0.20	3.19 ±0.17	3.33 ±0.18

[a]Mean±SEM, Student's 't' test; *P<0.05; **P<0.01; ***P<0.001

After continuous gamma-HCH feeding for 15 days (Figs. 13 to 22), the hepatic lobular structure remained unchanged during initial period of the experiment. The nuclei were compact with distinct vacuolar spaces which appeared quite prominent during the first 3 day treatment (Fig.14). The hepatic sinusoid showed slight dilation in 6 and 9 day treatments (Figs. 15 and 17) when compared with control (Fig.5). In low power objective, lightly and darkly stained areas can be differentiated (Fig.13). Any alteration in staining characteristics of the tissue is itself a pathological change. The cytoplasm was not granulated until day 15, when vacuoles appeared in the cytoplasm and slight granulation was visible (Figs. 22 and 28). Although the hepatic cells and nuclei appeared to be larger in size, but the typical indication of insecticide toxicity *i.e.*,

vacuolation is not distinctly visible in other treated groups. Irregular shaped condensed nuclei were frquently observed in the hepatic cells, in addition to normal nuclei. The number and size of kupffer cells also increased (Figs. 16-18). Almost similar changes were observed in 12 and 15 day gamma-HCH treated rat livers. The cytoplasmic margins were distinctly away from the nuclei, leaving clear spaces around them in 12 and 15 day treatment (Figs. 20 and 22). The clear areas around the nucleus may either be due to fatty degeneration or indicate hepatic glycogenesis.

Table III: Effect of feeding gamma-HCH mixed diet (9 mg/kg body weight/day) administered for 18 months on the various histological parameters.

Parameters	6 months gamma-HCH feeding experiment		12 months gamma-HCH feeding experiment		18 months gamma-HCH feeding experiment	
	Control	Treated	Control	Treated	Control	Treated
No. of cells/ field (n=9)	263.31[a] ±13.42	197.24* ±15.63	258.87 ±11.21	180.55** ±12.82	252.77 ±14.51	161.80** ±9.72
No. of nuclei/ cell (n=90)	1.14 ±0.09	1.32 ±0.26	1.19 ±0.13	1.40 ±0.30	1.12 ±0.11	1.27 ±0.07
No. of nucleoli/ nucleus (n=90)	1.25 ±0.05	1.38 ±0.07	1.28 ±0.07	1.50 ±0.17	1.21 ±0.47	1.30 ±0.10
Size of cell (μm^2; n=90)	272.41 ±9.13	365.34*** ±10.75	302.92 ±11.81	388.12** ±11.27	310.63 ±16.89	496.88** ±19.21
Size of nucleus (μm^2; n=90)	42.78 ±1.29	51.35** ±1.57	38.57 ±1.14	43.48 ±2.50	40.84 ±1.37	46.80* ±1.95
Size of nucleolus (μm^2; n=90)	2.88 ±0.31	4.97** ±0.25	2.64 ±0.41	3.74 ±0.35	2.71 ±0.19	3.72 ±0.33

[a]Mean±SEM. Student's 't' test: *P<0.05; **P<0.01; ***P<0.001

Figures 23 to 24 show the effects of lindane feeding for 6 months on the hepatic histological structure, while Figures 25-27 and Figures 28-31 indicate the effect of insecticide feeding in rat liver for 12 and 18 months, respectively. Although well defined liver cell hypertrophy alongwith condensed and hypertrophied nuclei can be observed (compare Figures 24 and 27 with Figure 7), the most prominent and significant change was noticed in 18 month group, where extensive cytoplasmolysis was seen with vacuolated and marginated cytoplasm. The nuclei of the hepatic cell were considerably condensed (Figs. 26, 27 and 29). Clear areas around nuclei were also visible in 12 and 18 month treatments (Figs. 25-27 and 29) which was an indication of slight cytoplasmic degeneration. The hepatic cord structure, generally, looks disturbed in long term study (Figs. 23, 25 and 28). The nuclear distortion (pycnosis) increased gradually in case of uninterrupted gamma-HCH feeding for 6-18 month period (Figs. 24, 27 and 29). The

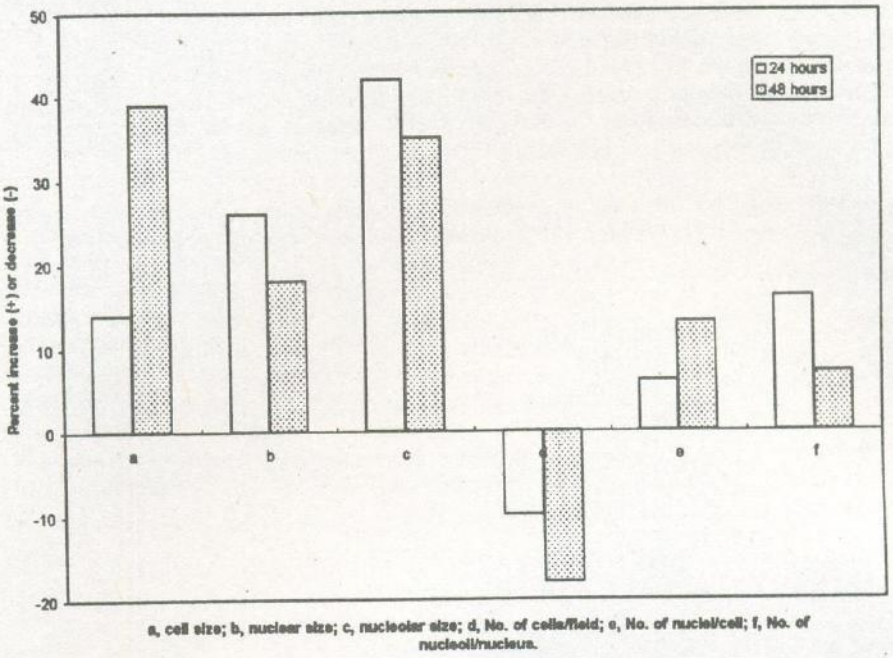


Fig.1: Percent increase or decrease in various morphometric and histological parameters of rat liver fed on lindane-mixed diet (30 mg/kg body wt./day) for total period of 48 hours.

liver of insecticide fed rats was marked by distended central vein, prominent blood vessels and bile canaliculi on the outer margins of hepatic cord cells during 18 month treatment (Figs. 28-29)

DISCUSSION

The size of hepatic cell, its nucleus and nucleolus increased considerably after lindane-treatment, the extent of which was highest in long term experiments. Increase in the size of above parameters has also been reported from different laboratories in various experimental animals by treating with other chemicals and organochlorine (OC) insecticides, like heptachlor, DDT, aldrin, dieldrin and endrin (Shakoori and Ali, 1986; Shakoori and Haq, 1987; Ali and Shakoori, 1990, 1996). Similar pattern of changes were also recorded in another study from this Lab in rats with chlorinated insecticide, endrin. According to this study the hypertrophy of the nuclei and nucleoli was due to the change in the fluid conntent of these organelles caused by changes in permeability of the nuclear membrane (Ali and Shakoori, 1993). Changes in permeability and other properties of the cell membrane may be responsible for induction of cellular hypertrophy.

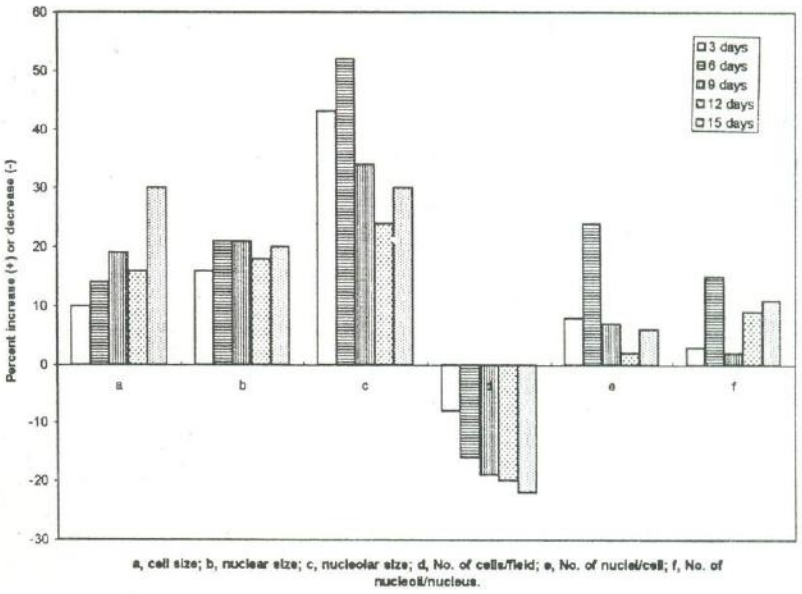


Fig.2: Percent increase or decrease in various morphometric and histological parameters of rat liver fed on lindane-mixed diet (18 mg/kg body wt./day) for total period of 15 days.

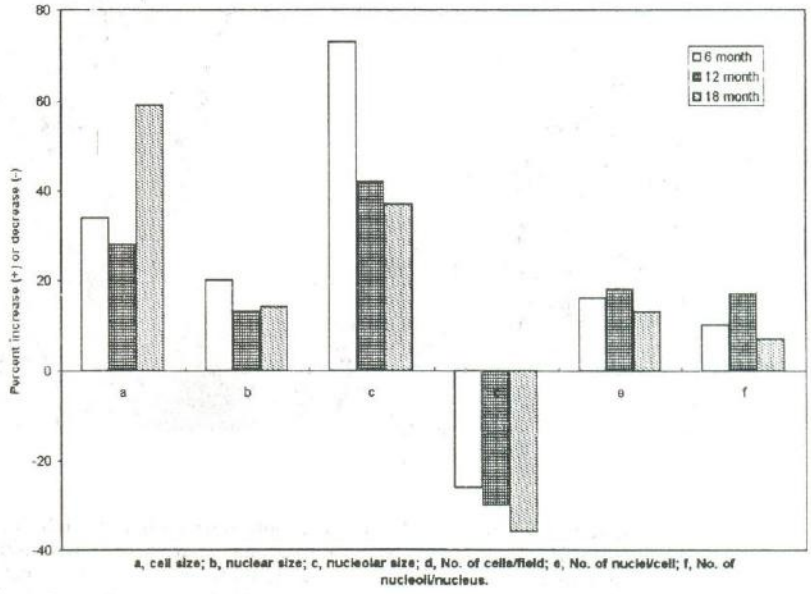
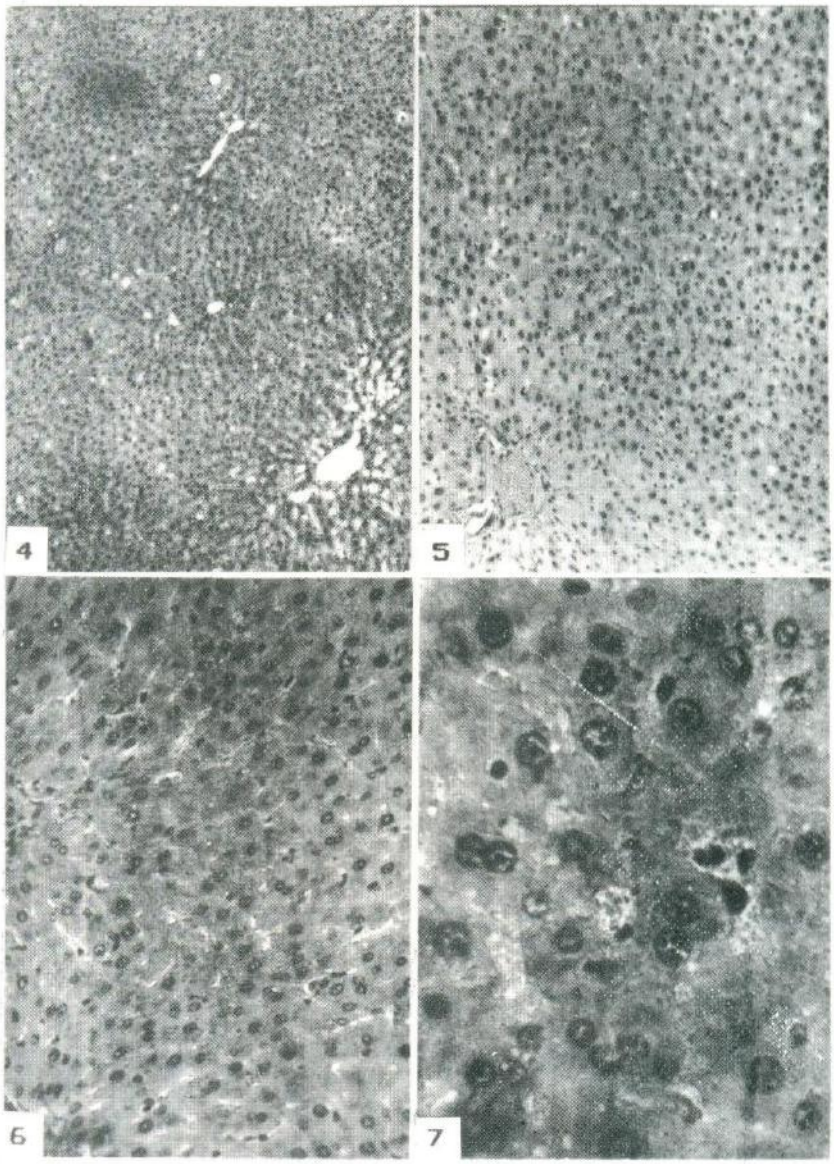
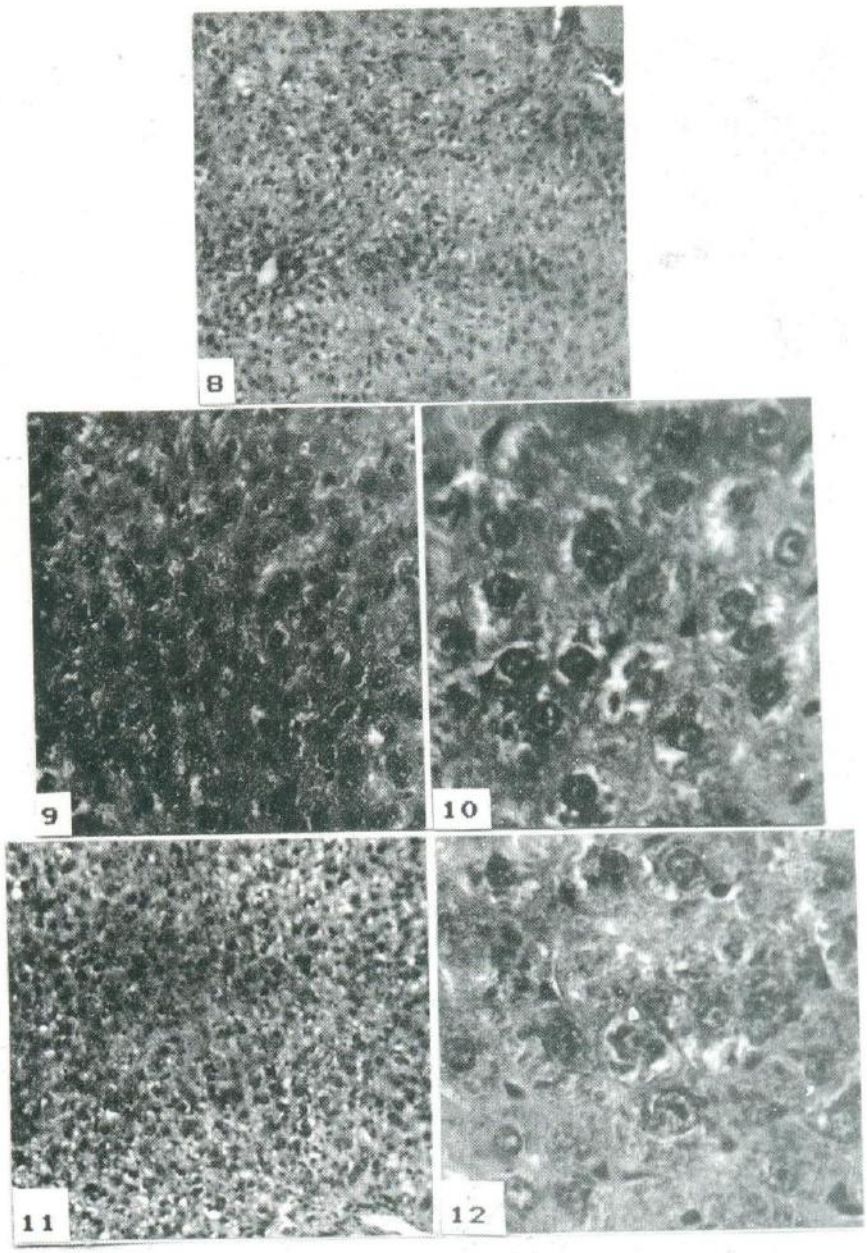


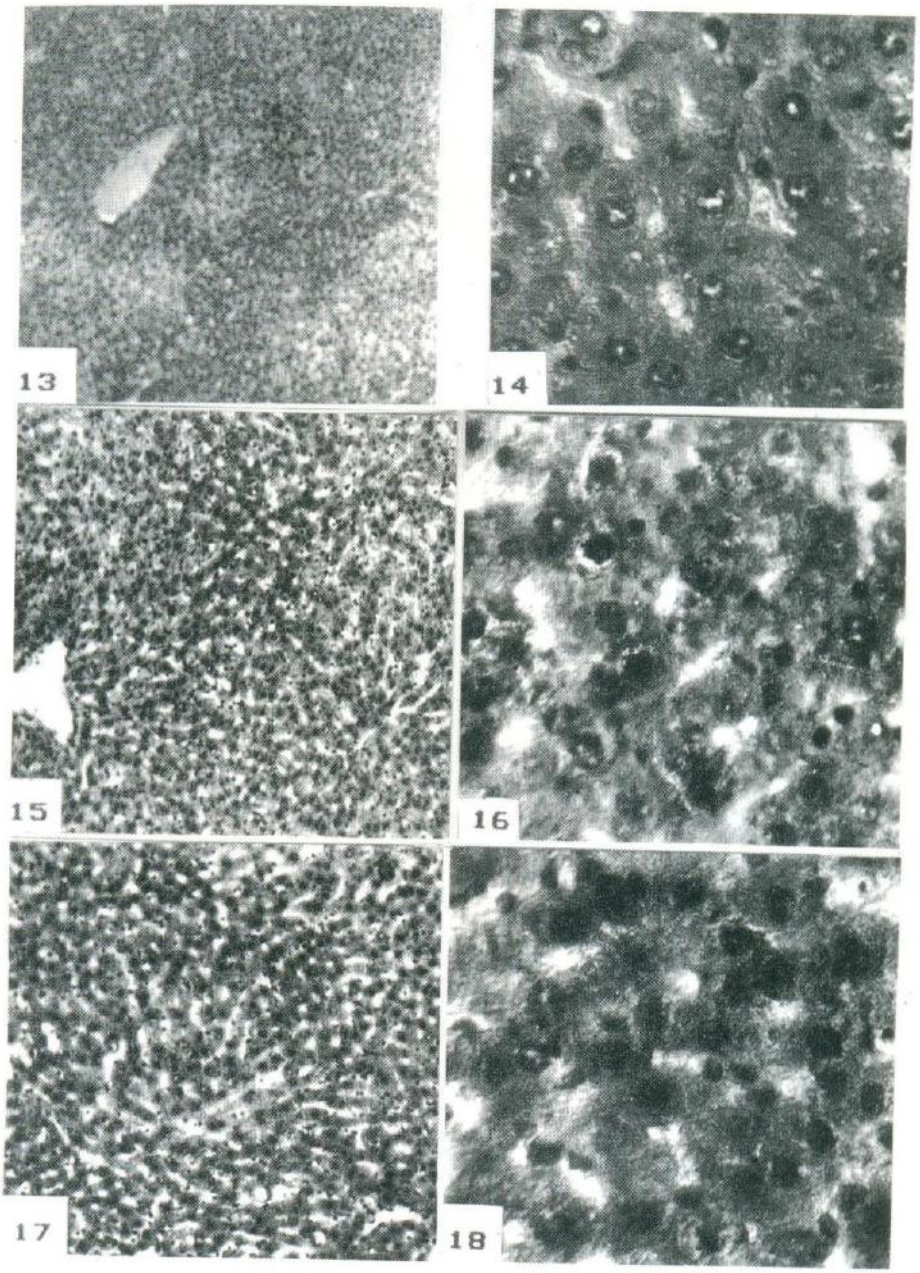
Fig.3: Percent increase or decrease in various morphometric and histological parameters of rat liver fed on lindane-mixed diet (9 mg/kg body wt./day) for total period of 15 days.



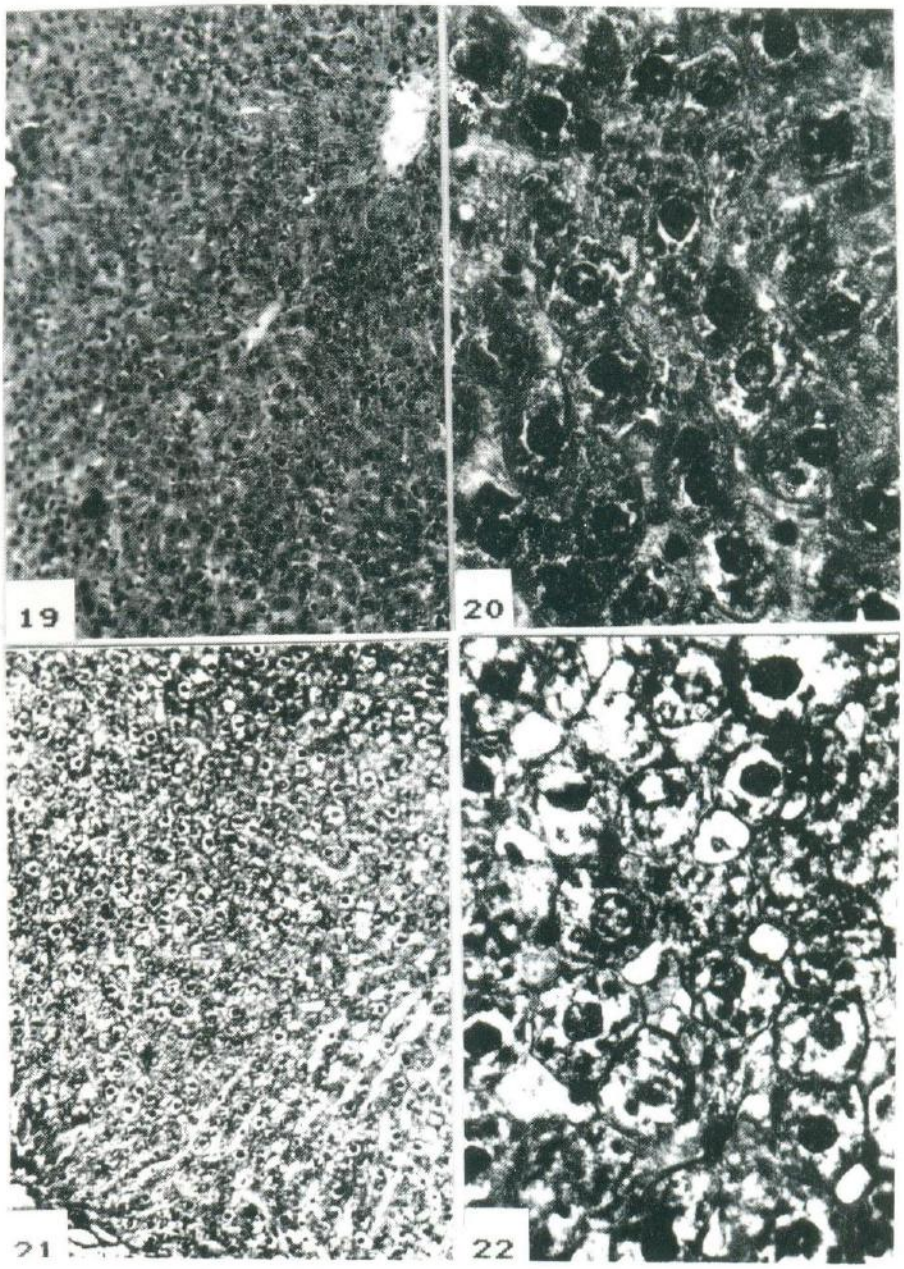
Figs. 4-7: Histological structure of normal rat liver. Note normal hepatic lobule, central veins with portal areas (Fig. 4-5), hepatic cord structure, sinusoidal spaces with rod shaped kupffer cells, liver parenchyma and nuclear arrangement (Figs.6-7). Stain: Haematoxylin and Eosin. Magnifications: Fig.4, 25X; 5, 50X; 6, 100X; 7, 250X.



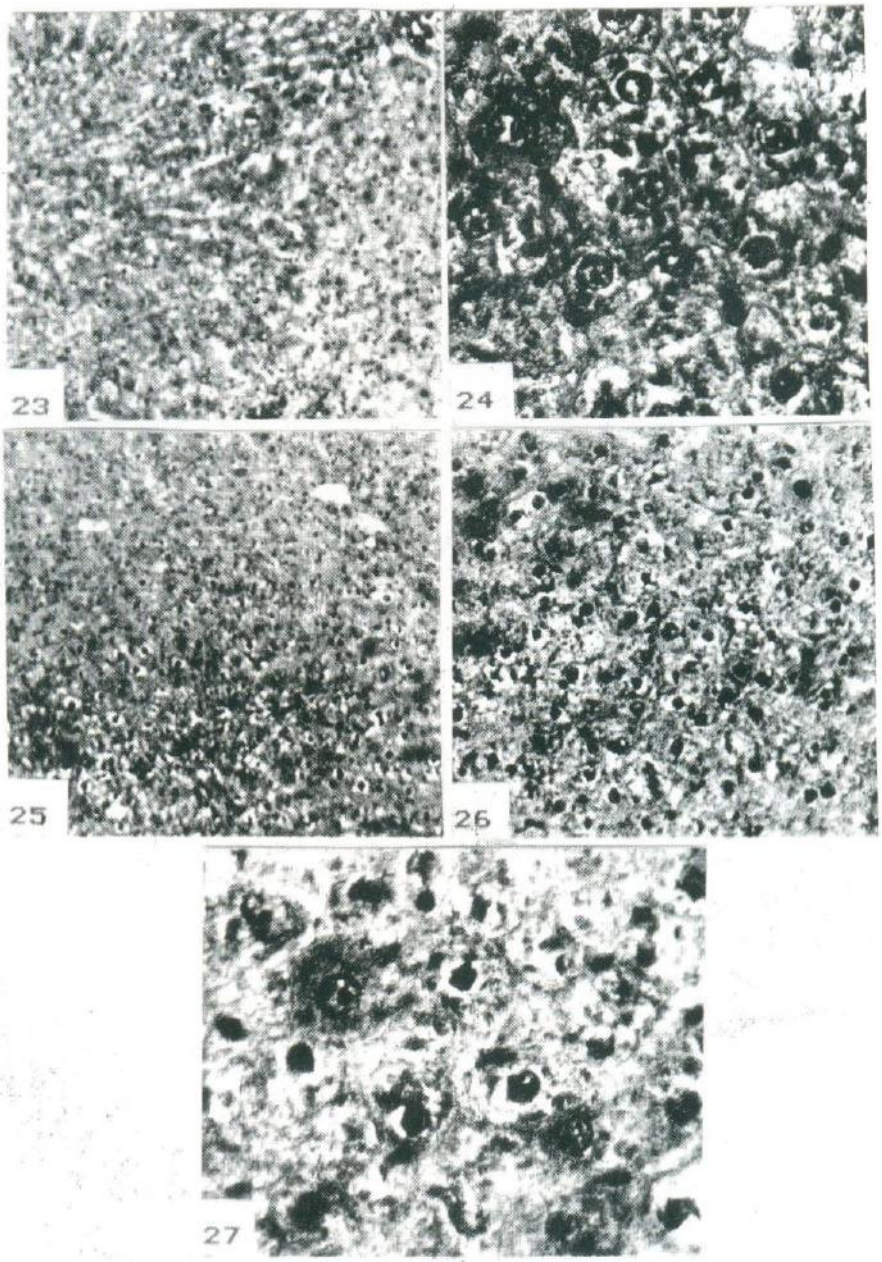
Figs. 8-12: Histological structure of rat liver fed on lindane-mixed diet for 24 hours (Figs.8-10) and 48 hours (Figs.11-12). Note disorganised lobular structure, and clear areas around the nuclei (Figs. 8 & 11) hypertrophied cells (Figs. 10 & 12)irregular shaped, picnotic nuclei (Figs. 9 & 11) and increase in clear areas (Figs. 10-11). Stain: Haematoxylin and Eosin. Magnifications: Figs. 8 & 11, 50X; 9, 100X; 10 & 12, 250X.



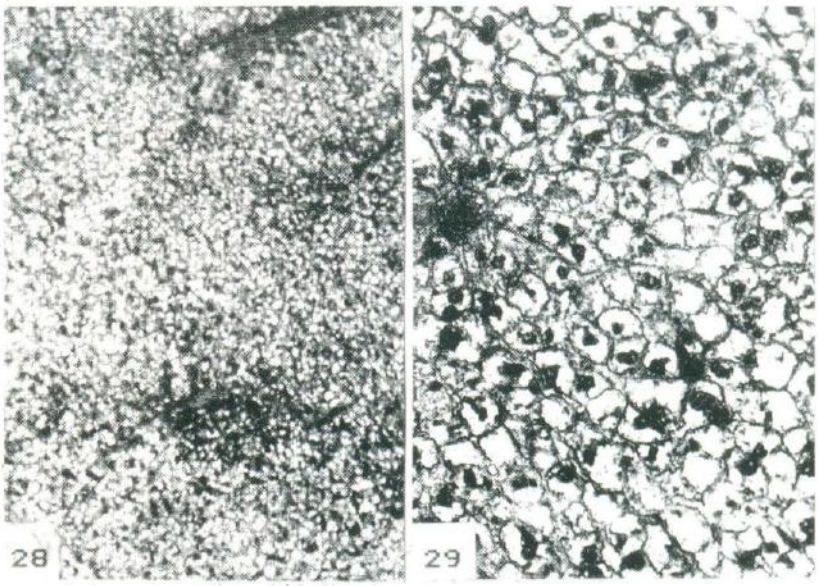
Figs.13-18: Histological structure of rat liver fed on lindane-mixed diet for 3 days (Figs. 13-14) 6 days (Figs. 15-16) and 9 days (Figs. 17-118). Note lobular structure with slightly disturbed cords (Figs. 13,15 & 17), lightly stained zones (Fig. 13), increased sinusoidal spaces and kupffer cells (Figs. 15-18), numerous clear areas around the nuclei (margination) and in the cytoplasm (Figs. 15 & 17), condensed, distorted, hyperchromatic nuclei (Figs. 16 & 18). Stain: Haematoxylin and Eosin. Magnifications: Figs. 13 & 15, 25X; 17, 50X; 14, 16 & 18 250X.



Figs.19-22: Histological structure of rat liver fed on lindane-mixed diet for 12 days (Figs. 19-20) and 15 days (Figs. 21-22). Note disturbed lobular architecture with disorganised regions (Figs. 19 & 21), hyperchromatic and picnotic nuclei with clear areas around them (Figs. 20-22)and fatty degeneration (Figs. 21-22). Stain: Haematoxylin and Eosin. Magnifications: Figs. 19 & 21, 50X; 20 & 22 250X.



Figs.23-27: Histological structure of rat liver fed on lindane-mixed diet for 6 months (Figs. 23-24) and 12 months (Figs. 25-27). Note totally disorganised regions of hepatic lobule with disturbed cord structure (Figs. 23, 25 & 26), dilated sinusoidal spaces (Fig. 23), hypertrophied cells and nuclei alongwith irregular shaped picnotic nuclei (Figs. 24-27), Also note fatty changes and clear areas (vacuolation) around the nuclei and in the cytoplasm (Figs. 23,26 & 27). Haematoxylin and Eosin. Magnifications: Figs. 23 & 25, 50X; 26, 100X; 24 & 27, 250X.



Figs.28-29: Histological structure of rat liver fed on lindane-mixed diet for 6 months (Figs. 23-24) and 12 months (Figs. 25-27). Note totally disorganised lobular structure with heavy vacuolation and almost invisible sinusoidal spaces (Fig. 29). Regions with variable staining (Fig. 28) thick membraned hepatic parenchyma alongwith picnotic nuclei (Fig. 29) were also evident. Haematoxylin and Eosin. Magnifications: Figs. 28, 25X, 29; 100X.

Increase in hepatic cell size during the present study, may be related to hypertrophy of the liver tissue which is a typical response of liver to foreign toxic compounds and most probably, is due to the rise in the relative weights of the liver (RLW), observed following exposure of animals to lindane and other OC insecticides (Shakoori *et al.*, 1984; Narayan *et al.*, 1990; Katayama, 1993; Ali and Shakoori, 1996). Prominent increase in the average size of the hepatic cell (15%) and its nuclei (10%) has been shown by oral feeding of a pyrethroid insecticide (cypermethrin) to rats @ 420mg/kg body wt./day (Shakoori *et al.*, 1988). The findings in the present series of experiments have also been supported by studies on aldrin feeding in rats for 18 month period (Ali and Shakoori, 1990). Chatervedi (1993) noticed a dose-dependent increase in liver/body weight ratio by 24-79 % in ICL male mice fed on mixture of 10 pesticides, including lindane (gamma-HCH), aldrin, dieldrin, DDT, endosulfan and alachlor. Liver cytochrome p-450 also increasec after gamma-BHC treatment @ 20 ppm for 15 and 30 days in the rats which in fact, is a consequence of raised smooth endoplasmic reticulum in the hepatic cell (Barros *et al.*, 1991). Drug mtabolism is one of the major functions associated with smooth endoplasmic reticulum and is carried out through induction of mixed-function oxygenase system utilizing cytochrome P-450 mediated pathway. Mikol *et al.* (1980) reported hepatic enzyme induction in rats by feeding gamma-HCH while raised synthesis of cytochrome P-450 content of the hepatic cell was shown by Kurihara *et al.* (1984).

Liver structure under light microscope revealed loss of cytoplasm and vacuolation at certain treatment durations. These findings also coincides with the study of Nigam *et al.* (1982) and Swaroop and Upadhyay (1985). Liver necrosis was also reported in rats by Barros and Saliba (1978). In another study, carbaryl has been shown to induce patchy lesions in the liver and developed endothelial damage to centro-lobular veins (Mari *et al.*, 1994). Various histological, histochemical and ultrastructural changes by pesticide exposure in animals have been reported from different labs (Zufarov *et al.*, 1975; Dikshith *et al.*, 1978a,b; Jeanne, 1979; Shivanandappa and Krishnakumari, 1981; Gupta and Singh, 1982, Nigam *et al.*, 1982, 1984a,b; Qin *et al.*, 1982; Preza *et al.*, 1983; Akhtar *et al.*, 1996; Ito *et al.*, 1996). Extensive accumulation of glycogen in liver (glycogenesis) of mice was reported after gamma-BHC feeding for 10 months (Nigam *et al.*, 1984a).

Rivett *et al.* (1978) however could not find any detectable histopathological changes after treatment of dogs with 100 and 200 ppm of lindane for 104 weeks. The degenerative changes in liver after 12 weeks of lindane feeding were reported by Su and Zhou (1986) and according to this report lindane induced granular hyaline degeneration of hepatic tissue. Lindane-induced peripheral necrosis with haemorrhagic foci in the liver of rat were shown by Junqueira *et al.* (1991). Gamma-HCH also induced injury and lesions in other tissues. Baronia and Sahai (1990) observed lindane-induced adrenal pathology in rats, with necrotic changes, ruptured cells, pycnotic nuclei and increase in spaces and vacuolation at 200mg/kg body weight dose level.

Fatty changes, degeneration, necrosis, cytoplasmic vacuolation and lipid peroxidation are the other pathologies observed in the hepatic and renal tissues of rats, mice guinea pigs and hamsters with oral administration of OC insecticide, endrin, alongwith the corn oil (Hassan *et al.*, 1991).

Lindane also stimulates hyperplasia (cell proliferation) in the liver (Brade *et al.*, 1974). Hyperplasia normally occurs due to increased mitotic activity which, in turn, is a consequence of increased DNA synthesis in the tissue (Yusof and Edwards, 1990;

Waller *et al.*, 1996; Rought *et al.*, 1998). This observation was further confirmed by the findings of another part of this study (Ali, 1988) in which it has been noted that gamma-HCH produced 123% and 55% increase in DNA of rat liver after 15 days and 18 month experiments, respectively. Development of liver and other tissue cancer has been reported in different animals (Ito *et al.*, 1975; Herbst, 1975; Reuber, 1979; Kashyap *et al.*, 1979; Bhatt *et al.*, 1981d; IARC, 1982; Nigam *et al.*, 1982, 1984; Oesch *et at.*, 1982; Ahlborg *et al.*, 1995; Ito *et al.*, 1996; Rosa *et al.*, 1996) but in the present study we could not find any malignant change in rat liver.

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