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Original Article

LOW DOSE HETEROGENEOUS CHEMICAL MIXTURE EXPOSURE INDUCED ALTERATIONS IN THE STRUCTURE AND ANTIOXIDANT DEFENSE SYSTEM OF RAT LIVER

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Abstract: Adult male rats were exposed to heterogeneous chemical mixture (HCM) of Phthalic acid di butyl ester, 1, 2 –Dichlorobenzene, Cadmium chloride and Chromium trioxide, through oral gavage, at a dose level equal to 1/100 and 1/1000 of LD_{50} value of each chemical compound for 60 days followed by withdrawal of exposure until 120 day of experiment. Histological alterations, serum parameters of liver function and oxidative stress parameters in liver assessed the status of structure and function of liver following exposure. The increased serum liver marker enzyme activities and decreased endogenous antioxidant levels suggested that HCM exposure below individual chemical's NOAEL doses significantly hamper the normal function of liver. This is well correlated with increased CHO, LDL and VLDL level because these appear harmless until they are in blood vessel walls and oxidised by free radicals. Thus, HCM exposure has a definite effect on liver structure and function. However, the low dose HCM induced changes are reversible and effectively reversed by withdrawal of treatment for comparatively longer duration of time.

Key words: Industrial chemical mixtures, Liver

INTRODUCTION

Simultaneous exposure to more than one chemical may modify the biological activity of other chemicals and such interaction might result in potentiating, decreasing or nullifying the ultimate effect of chemical mixture. The majority of existing toxicological data describe toxicity of single compound or a mixture of few chemicals of the same classes, which does not assess the toxic potentials of chemical mixtures [1-3]. Environmentally persistent chemicals are consistently found as contaminant in air, water and food sources and through the food chain consequently in human blood, milk, urine and hair samples [4]. However, the detected concentrations of individual toxicants in humans are generally below the levels shown to cause adverse effects in laboratory animals [5,6]. Moreover, the individual substance toxicity studies in animals evaluate the toxic responses at relatively higher dosage and shorter duration of exposure as compared to the ambient exposure of humans [7,8]. Epidemiological or clinical evidences are insufficient to confirm that experimentally proven carcinogenic substances cause cancer in humans at detected environmental levels. Further, epidemiology of multiple chemical exposures is a relatively unexplored field in occupational and environmental health. Recently, survey of chemical body burden in US population noted presence of 212 chemicals in the blood and/or urine samples where 75 of these compounds were recorded for the first time; suspecting total number

of pollutants/ chemicals much higher than detected [9,10]. The use of combination drugs, herbal extracts in therapeutics, cosmetics etc. has increased in past two decades, which may result into low level body burden of heterogeneous chemical mixture with great potentials to interact with already existing toxicant burden [11].

In present investigation, the heterogeneous mixture was assessed for its potentials of inducing structural and functional alterations in the liver of the rat. The test chemicals are environmentally persistent constituents of the discharges from various industries of central and south Gujarat that are released into the Gulf of Khambhat, Gujarat, India and thus pollute various resources and pose health hazards [12,13]. The test mixture included phthalic acid di butyl ester, 1,2–dichlorobenzene, cadmium chloride and chromium trioxide, administered through oral gavage at low doses for 60 days following withdrawal for next 60 days.

Present experiment demonstrates that very low level of heterogeneous chemical mixture exposure can be potentially toxic. Since toxicity of individual component of the mixture is studied in detail for their dose-response relationship and potential hazards, retesting of the individual component toxicity was not required and therefore, not carried out.

MATERIALS AND METHODS

Animals: Adult male Wistar rats (300-350g), were acclimatized in departmental animal house for 15 days in standard conditions (22±3°C, L:D 12:12). They were fed with commercial rat chow and water ad libitum. The experimentation on animal was approved by Institutional Animal Ethical Committee; animal handling and all procedures on animals were carried out in accordance with the guidelines.

Chemicals: The chemicals (SISCO Research Laboratories, Gujarat) used in the study were of analytical grade or of the highest grade commercially available. Phthalic acid di butyl ester and 1,2–dichlorobenzene were mixed in corn oil while cadmium chloride and chromium trioxide were dissolved in distilled water at a concentration equal to 1/100 and 1/1000 of LD50 value of individual chemical. The administered doses are lower than the Non observable Adverse Effect Level of individual experimental chemical [14-17] (Table 1). At the time of dosing all the toxicants were mixed to a total volume of 0.8 ml and administered through oral gavage.

Experimentation for toxicity and withdrawal study: Each group comprised of five rats. Rats of group I (day zero control group) were sacrificed on day 1 (Table 1). Rats of day 60 and day 120 control group (Gr. II and VI, respectively) were maintained on normal diet. Rats of day 60 vehicle control group (Gr. III) were administered 0.4 ml of corn oil mixed with 0.4 ml of water. Rats of day 120 vehicle control group (Gr. VII) were administered vehicle similarly for 60 days followed by no treatment during the withdrawal phase until day 120 (Table 1). Group IV (low dose HCM- day 60) and Group V (high dose HCM- day 60) rats were exposed for 60 days to chemical mixture (Phthalic acid di butyl ester, 1, 2 – dichlorobenzene, cadmium chloride and chromium trioxide) at a dose level equal to 1/100 and 1/1000 of LD50 value of each chemical compound, respectively. Rats of 120 day toxicant group (Gr. VIII: Low dose HCM and Gr. IX: High dose HCM) were administered the toxicant mixture as above for 60 days followed by withdrawal of administration until day 120 (Table 1).

Analytical procedures: Blood samples were collected from retro-orbital sinus plexus to obtain serum. The serum samples were stored at -40C until assayed. Clinical chemistry determination was performed by using semi-automatic biochemistry analyzer (ERBA CHEM-5 Plus). All the parameters were analyzed using commercially available kits.

Liver function tests: Activity of enzyme and concentration of certain biochemical parameters were determined in serum as follows: serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase activities (SGPT) and lactate dehydrogenase (LDH) were determined according to the method described by International Federation of Clinical Chemistry (IFCC using kits from Transasia Biomedicals Ltd., India). Gamma glutamyl transpeptidase (GGT) and Alkaline phosphatase (ALP) activity were measured using kit purchased from Transasia Biomedicals Ltd., India and Reckon Diagnostic Pvt Ltd., India respectively. Total protein and albumin were determined by using

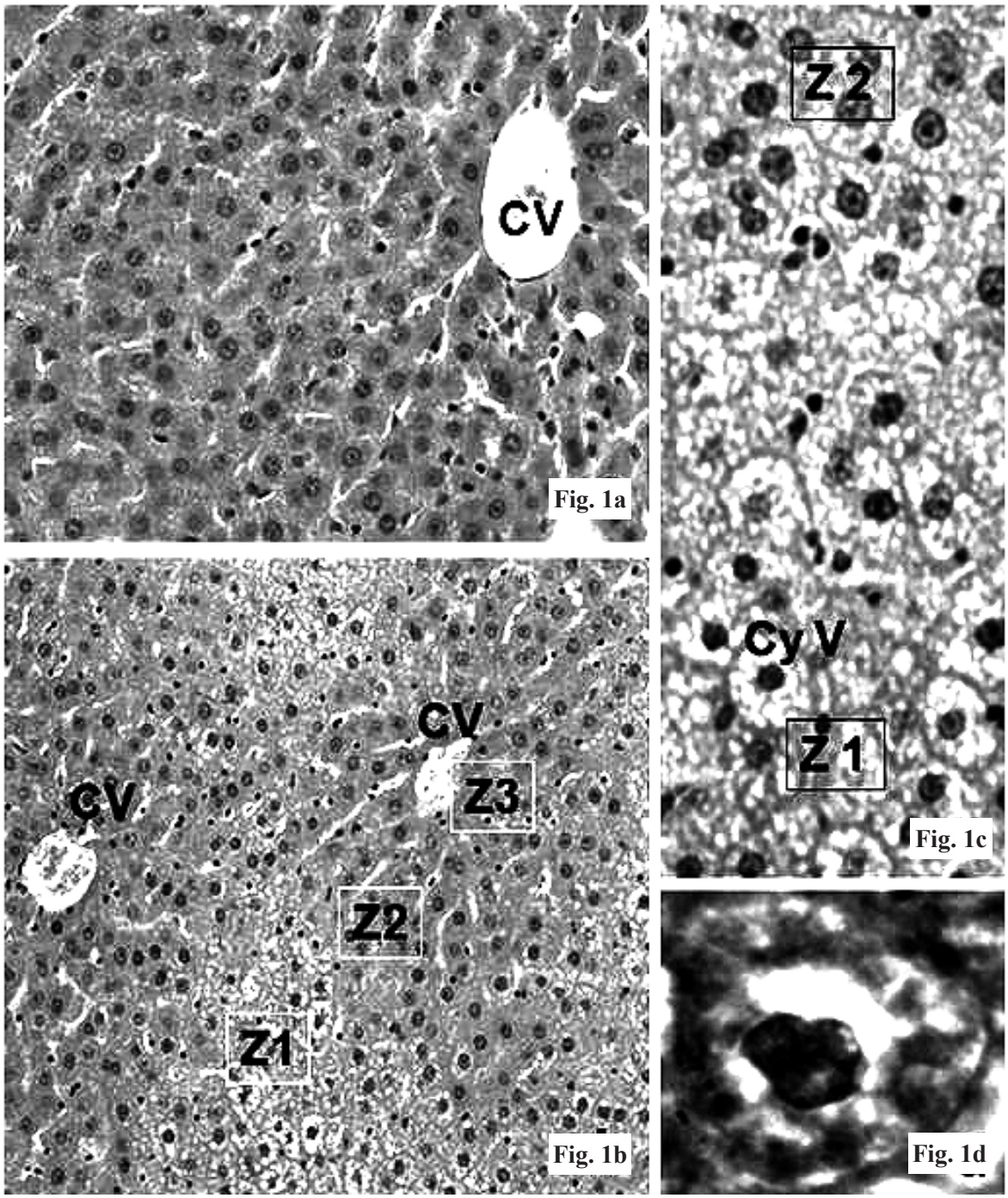

Fig. 1: Liver sections of rat stained with haematoxline and eosin. Fig. 1a: Showing parenchymatous appearance of the liver of control animal. The hepatic cords are arranged along the central vein (CV) and have distinct sinusoidal spaces. The nuclei of hepatocytes are prominent, Kupffer cell are lined along the sinusoids 200X. **Fig. 1b:** The cells of Zone-1 are highly vacuolated, that of Zone-2 moderately affected and that of Zone-3 least vacuolated. The cytoplasm of Zone-3 hepatocytes are comparatively more eocinophilic. 200 X. **Fig. 1c:** Showing the pattern and extent of cytoplasmic vacuolation particularly in Zones 1 and 2. The hepatocytes are swollen and hence the sinusoidal spaces are constricted. Marginal hyperplasia of Kuffer cell is clear. 400X. **Fig. 1d:** Prominent nuclear changes noted in the toxicant mixture exposed. 1000X.

Table 1: Experimental protocol

Group	Treatment	Autopsy day
I	Zero (initial) day control	01
II	Control (No treatment)	61
III	Control (Vehicle administration for 60 days)	61
IV	Heterogeneous Chemical Mixture High Dose treated group (1% of LD_{50} dose/ animal/ day) (administered for 60 days)	61
V	Heterogeneous Chemical Mixture Low Dose treated group (0.1% of LD_{50} dose/ animal/ day) (administered for 60 days)	61
VI	Control (No treatment)	121
VII	Control (Vehicle administration for 60 days followed by no treatment further till 120 days)	121
VIII	Heterogeneous Chemical Mixture High Dose treated group (1% of LD_{50} dose/ animal/ day) for 60 days followed by withdrawal phase until 120 days.	121
IX	Heterogeneous Chemical Mixture Low Dose treated group (0.1% of LD_{50} dose/ animal/ day) for 60 days followed by withdrawal phase until 120 days.	121

Table 2: Serum markers of liver function following exposure to heterogenous chemical mixture. Values are Mean± SE and statistical significance is compared with respective vehicle control, (*) P<0.05, (++) P<0.01 and (#) P<0.001.

Gr.	SGPT (U/L)	SGOT (U/L)	GGT (U/L)	ALP (U/L)	LDH (U/L)	Bilirubin (mg/dL)
I	34.44 ±2.75	81.43 ±2.79	7.36 ±0.40	98.87 ± 3.46	1352 ±51.28	0.33 ±0.15
II	39.63 ±2.42	85.86 ±3.16	7.24 ±0.35	104.80 ±4.90	1534 ±31.42	0.12 ±0.02
III	37.22 ±2.10	80.71 ±2.31	8.62 ±0.43	106.40 ±4.26	1410 ±21.45	0.15 ±0.02
IV	50.49 ±3.30*	105.80 ±3.80^{++}	12.47 ±0.84$^{\#}$	125.40 ±7.86	1653 ±42.30^{++}	0.24 ±0.04
V	47.41 ±3.91	108.40 ±4.32$^{\#}$	10.49 ±0.58	119.70 ±5.46	1600 ±17.36*	0.20 ±0.05
VI	37.55 ±1.17	93.67 ±4.47	9.38 ±0.29	112.90 ±3.29	1408 ±47.83	0.09 ±0.01
VII	33.62 ±3.07	95.00 ±3.88	9.58 ±0.39	109.50 ±2.51	1411 ±23.09	0.11 ±0.02
VIII	43.45 ±1.09	115.00 ±4.36*	10.18 ±0.47	131.40 ±6.80	1587 ±22.73*	0.13 ±0.04
IX	47.45 ±1.33*	106.80 ±5.34	9.45 ±0.42	130.10 ±6.61	1493 ±51.99	0.09 ±0.01

Table 3: Serum lipid profile as marker of liver function following exposure to heterogenous chemical mixture. Values are Mean± SE and statistical significance is compared with respective vehicle control, (*) P<0.05, (++) P<0.01 and (#) P<0.001.

Gr.	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-cholesterol (mg/dl)	LDL- cholesterol (mg/dl)	VLDL- cholesterol (mg/dl)
I	78.67 ±2.32	52.29±2.44	42.15±1.13	26.06±2.27	10.46±0.49
II	80.51±2.69	55.11±2.65	46.11±0.97	23.37±3.39	11.02±0.53
III	83.17±2.60	56.36±2.83	44.65±1.70	27.25±3.39	11.27±0.57
IV	108.30±3.52$^{\#}$	69.21±1.29*	41.17±1.14	53.27±4.26$^{\#}$	13.84±0.26*
V	96.45±3.13*	58.50±1.61	43.23±1.48	41.52±3.23	11.70±0.32
VI	86.22±2.40	53.43±2.70	40.51±1.21	35.02±1.79	10.69±0.54
VII	85.13±2.45	51.78±2.36	43.47±2.30	31.30±3.90	10.36±0.47
VIII	88.10±3.07	51.06±3.18	39.10±3.27	38.79±4.95	10.21±0.64
IX	82.15±2.32	49.66±2.78	42.20±1.80	30.02±4.00	9.93±0.55

Table 4: Enzymes and metabolites of general liver function following exposure to heterogenous chemical mixture. Values are Mean± SE and statistical significance is compared with respective vehicle control, (*) P<0.05, (++) P<0.01 and (#) P<0.001.

Gr.	SDH (µg formazan formed/ mg protein)	ACP (µ moles of p-nitrophenol released/ mg protein/ min)	ALP (µ moles of p-nitrophenol released/ mg protein/ min)	Cholesterol (mg/ 100 mg tissue)
I	18.65 ±2.21	0.26 ±0.011	0.12 ±0.007	0.44 ±0.02
II	21.71 ±2.63	0.28 ± 0.009	0.13 ±0.003	0.46 ±0.03
III	20.35 ±0.97	0.25 ±0.013	0.11 ±0.005	0.50 ±0.04
IV	11.67 ±0.71*	0.23 ±0.019	0.10 ±0.007	0.28 ±0.03^{++}
V	19.25 ±1.23	0.21 ±0.014	0.11 ±0.007	0.43 ±0.03
VI	18.73 ±1.51	0.26 ±0.010	0.13 ±0.008	0.44 ±0.02
VII	18.29 ±1.94	0.26 ±0.018	0.14 ±0.010	0.54 ±0.03
VIII	26.66 ±1.03*	0.28 ±0.017	0.14 ±0.012	0.47 ±0.04
IX	27.75 ±1.29^{++}	0.21 ±0.019	0.11 ±0.010	0.40 ±0.07

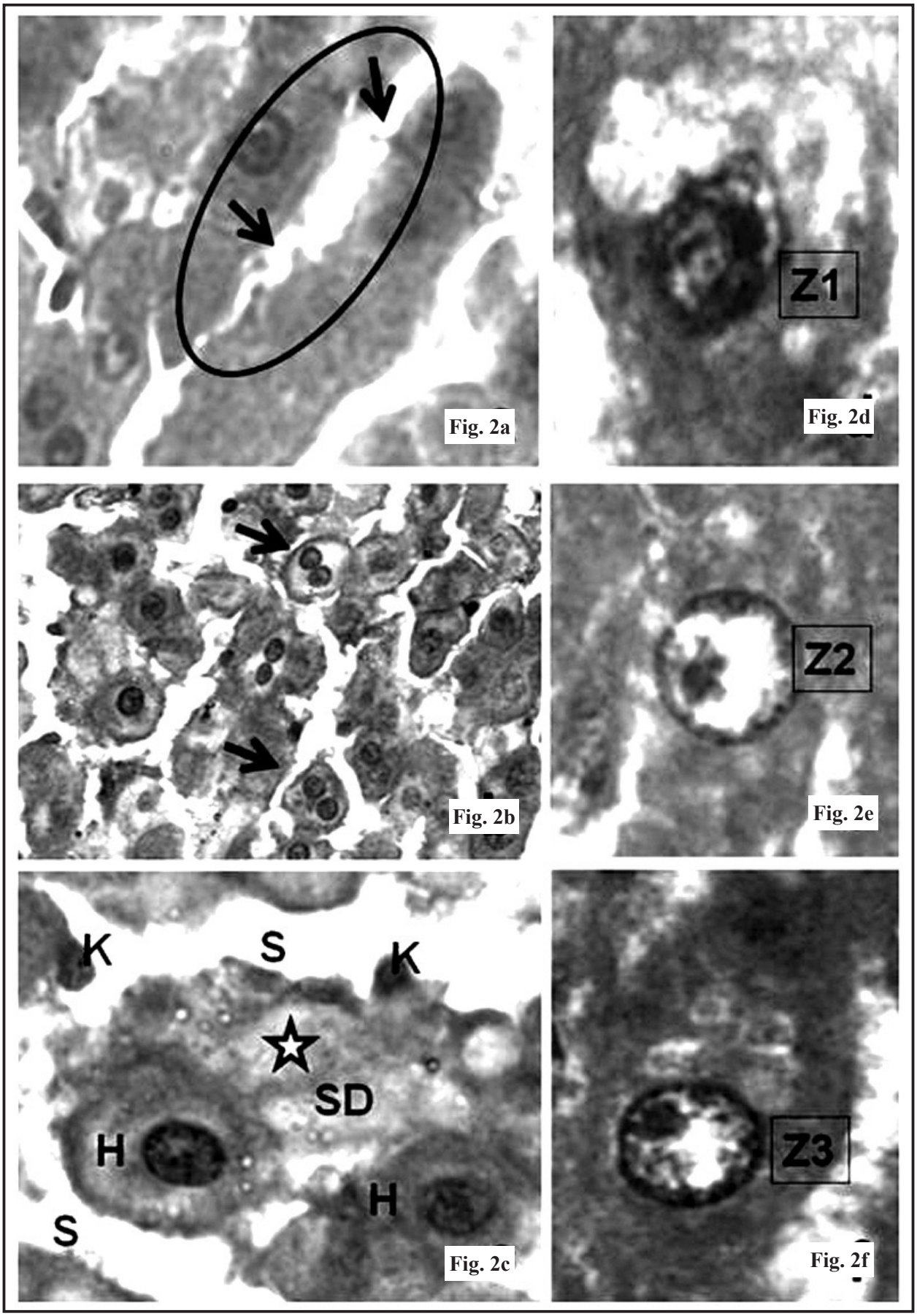
Fig. 2a
Z1
Fig. 2d
Fig. 2b
Z2
Fig. 2e
K
S
K
SD
H
H
S
Fig. 2c
Z3
Fig. 2f

Table 5: Oxidative stress indicators of liver function following exposure to heterogeneous chemical. mixture. Values are Mean± SE and statistical significance is compared with respective vehicle control, (*) P<0.05, (++) P<0.01 and (#) P<0.001.

Gr.	Protein (mg/ 100mg tissue)	LPO (n mole MDA/ 100mg issue)	GSH (µg of GSH/ mg protein)	Ascorbic acid (µg/ mg tissue)	SOD (Unit/ mg protein)	GPx (µg of GSH utilize/ min/ mg protein)	CAT (µ mole of H_2O_2 consumed/ min/ mg protein)	GST (µ mole CDNB-GSH conjugate formed /min/ mg protein)
I	21.04 ±0.85	79.04 ±7.01	6.66 ±0.56	0.46 ±0.05	13.19 ±0.97	10.59 ±0.65	58.96 ±2.68	7.41 ±0.45
II	20.79 ±0.52	77.42 ±7.67	6.79 ±0.44	0.50±0.06	12.04 ±1.21	9.89 ±0.67	63.81 ±5.82	8.50 ±0.39
III	21.75 ±0.60	71.90 ±5.71	7.07 ±0.63	0.49±0.07	13.35 ±0.45	9.41 ±0.53	68.16 ±8.03	8.01 ±0.42
IV	17.77 ±0.37*	111.00 ±5.17^{++}	3.09 ±0.40$^{\#}$	0.22 ±0.03*	8.18 ±0.77*	6.22 ±0.46	39.61 ±2.43^{++}	3.29 ±0.20$^{\#}$
V	19.14 ±0.96	102.70 ±2.14*	4.43 ±0.26*	0.22 ±0.03*	10.19 ±1.32	8.25 ±0.72	38.41 ±3.03^{++}	4.65 ±0.55^{++}
VI	21.20 ±0.90	87.20 ±9.74	7.14 ±0.55	0.47±0.06	13.89 ±0.88	9.96 ±0.70	62.82 ±5.17	8.51 ±0.73
VII	21.31 ±1.02	89.07 ±4.38	6.49 ±0.56	0.39±0.05	11.01 ±1.17	10.13 ±0.69	58.99 ±3.39	9.04 ±0.64
VIII	19.23 ±1.00	99.26 ±5.79	6.81 ±0.50	0.37±0.07	14.27 ±0.89	11.72 ±0.88	36.17 ±4.03*	6.24 ±0.65*
IX	20.03 ±0.57	114.2 ±7.58	7.28 ±0.55	0.36±0.06	12.98 ±0.67	9.84 ±0.76	48.15 ±4.59	7.26 ±0.64

standard kits from Beacon Diagnostics Pvt Ltd., India. Globulin concentration was calculated by subtracting albumin level from total protein. Serum bilirubin concentrations were estimated by using kit from Bayer Diagnostic India Ltd.

Lipid profile: Total cholesterol (TC) and triglyceride (TG) were estimated by enzymatic method described in the kit purchased from Eve's Inn Diagnostics, India and Bayer Diagnostic India Ltd, respectively. High density lipoprotein levels were estimated using Transasia Biomedicals Ltd., India kits, low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) calculated as per Friedwald's equations: VLDL-c = TG/5 and LDL-c = TC-[HDL-c+ (TG/5)].

Following blood collection, the animals were sacrificed for tissue collection. The mid lobe of liver was excised, blotted free of blood, weighed and utilized for histological examination and biochemical estimations.

Biochemical estimations in liver: The level of lipid peroxidation (LPO) was measured as the malondialdehyde (MDA) content [18]. The protein [19] and reduced glutathione (GSH) [20] content and estimation of activities of superoxide dismutase [21], catalase (CAT) [22], glutathione peroxidise (GPx) [23] and glutathione- S-transferase (GST) [24] were assayed by standard methods.

Histological examination: The mid lobe of liver was dissected out and fixed in Bouin's fixative. The tissues were processed for paraffin embedding and 5 µm sections were stained with hematoxylin and eosin for microscopic examination.

Statistical analysis: All the data are expressed as Mean ± Standard Error. The statistical analysis of the data was done using one way ANOVA followed by Bonferroni comparison test using Graph Pad Prism. All statistical tests were run at 95 % confidence interval and P d" 0.05 was taken as the level of statistical significance. Statistical comparisons were made between HCM treated and vehicle control/ control group on respective days.

RESULTS

No significant changes were observed in absolute and relative liver weight at any dose of Heterogeneous Chemical Mixture (HCM) and the weight remained within the normal range.

Explanation to figures:

Fig. 2: Liver sections rat exposed to high doses of HCM for 60 days stained with haematoxline and eosin (Fig. 2a). Fig. 2b-f: liver of rats exposed to high doses of HCM for 60 days followed by withdrawal of further 60 days (total 120 days).

Fig. 2a: Showing the prominent features of cell damaged to hepatocytes. The cells are swollen. Cytoplasmic blebbing is prominent (arrow) and the cell exudates in the sinusoids are also seen 650X. Fig. 2b: Several binucleate hepatocytes are seen where in some of the case cytoplasm is also normal. This is generally considered as a feature of hepatic regeneration 400X. Fig. 2c: The exudates leaked from the hepatocytes are present in the extensively extended space of disse seen between the hepatocytes and the margin of the sinusoid. The kupffer cells lining the sinusoids are also clearly seen 400X. Figs. 2d,e,f: The nuclei of the hepatocytes from the different zones of acinus appear quite normal. However, cytoplasmic changes, especially vacuolation, are prominently seen. It was more conspicuous in Zone 1 followed by Zone 3 and Zone 3, respectively. HE, 1000X

Effect on liver functions: The effects of HCM administration on liver marker enzymes and total bilirubin contents are presented in Table 2. Activities of SGPT and GGT increased significantly following high dose HCM exposure to rats for sixty days. Low dose HCM treatment also caused increase in the activity of SGPT and GGT enzymes but the elevation was insignificant when compared with controls. The activities of SGPT and GGT restored towards the normal value in withdrawal groups (VIII and IX) when compared to rats exposed to HCM (group IV and V). However, the enzyme (SGPT and GGT) activities remained high in recovery groups as compared to their controls. The activity of serum SGOT showed a significant ($P<0.01$ and $P<0.001$) increase in high and low dose HCM treated rats compared to vehicle treated control group and also remained significantly ($P<0.05$) high in recovery group VIII (high dose HCM + recovery). Insignificant elevated activity of ALP was observed in the HCM exposed groups (Table 2). Similarly, no significant change in the level of total bilirubin was observed in any of the HCM treated groups. The activity of LDH showed a significant ($P<0.01$ and $P<0.05$) increase in HCM (high and low dose respectively) treated rats when compared to vehicle treated control. Here again the activity of LDH restored towards the normal value in withdrawal groups (VIII and IX) when compared to rats exposed to HCM (group IV and V). Nevertheless, activity remained significantly high in groups VIII (high dose HCM + recovery) when compared with control group VII (Table 2).

Table 3 shows the significant increase in serum cholesterol (CHO), triglyceride (TG), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) in group IV (high dose HCM) as compared to group III (vehicle treated group). The low dose HCM treatment caused only significant increment in CHO level while the elevated difference in TG, LDL and VLDL level were insignificant with control group. The level of CHO, LDL and VLDL were reduced in group VIII and IX i.e. elevated level reduced after withdrawal from HCM exposure. The variation in high density lipoprotein (HDL) level was non significant in HCM exposed as well as in recovery groups (Table 3).

Table 4 shows the activities of succinate dehydrogenase (SDH), acid phosphatase (ACP) and alkaline phosphatase (ALP) and cholesterol level in liver of rats exposed to HCM. Treatment with high dose HCM for sixty days caused significant ($P<0.05$) decrease in the activity SDH and its significant ($P<0.05$) increase, in recovery groups (VIII and IX). Treatment with low dose HCM caused no significant change in the activity of SDH enzyme in liver. However, there was no significant change in the ACP and ALP enzyme activities. Treatment with high dose HCM caused significant ($P<0.01$) decrease in the liver cholesterol level (Table 4).

Protein content of liver decreased in sixty days HCM exposed groups (Table 5). The decrease was statistically significant in high dose HCM treated group IV, as compared with vehicle treated control group III. Withdrawal of the treatment for another sixty days showed recovery towards normalization. Administration of HCM caused changes in the levels of LPO (Table 5). LPO levels were found to be significantly elevated in high and low dose HCM exposed rats when compared with vehicle treated control rats. The elevated LPO level restored toward the normal values in recovery groups VIII and IX when compared with HCM exposed and control groups. The results of oxidative stress parameters revealed that the administration of HCM caused significant damage as evident by marker enzymes of antioxidant defense system. Table 5 depicts that the content of non enzymatic antioxidants such as GSH, ascorbic acid and activities of enzymatic antioxidants such as SOD, CAT and GST declined significantly upon HCM treatment (group IV and V) when compared with vehicle treated control (group III). No statistically significant differences were noted in the activity of GPx between the treated and control rats. Withdrawal of the HCM treatment for sixty days showed recovery in few antioxidant parameters, except CAT and GST in group VIII (high dose HCM treated + recovery), which were not recovered compared to 120 days control group VII (Table 5).

Effect on liver histoarchitecture: Structurally, the liver is divided into classical polygonal lobules. The hepatic acinus is physiologically more appropriate unit of liver. The acinus is located between two central veins and two portal triads encompassing the hepatic cords distributed in between. The acinus is divided into zones 1, 2 and 3 and the hepatocytes in these zones have different metabolic functions.

Zone 1 is closest to the portal tract and receives the most oxygenated blood, while zone 3 is furthest away closer to central vein. The major cell type of liver is polygonal hepatocyte are separated by capillary sinusoids. The sinusoids are lined by a discontinuous, fenestrated endothelium, devoid of basement membrane which is separated from the hepatocytes by a narrow space of Disse, which drains into the lymphatic of the portal tracts. This is also the region where from the exchange of material between the sinusoid and hepatocytes occur. The liver sinusoids receive blood from terminal branches of both the hepatic portal vein and hepatic artery. The Kupffer cells that form part of the monocyte-macrophage defense system are scattered along the lining of sinusoids.

In the control animals, the liver exhibited normal arrangement of hepatic cords and all the cell types (Fig. 1a). Administration of high doses of HCM for 60 days leads to moderate to severe changes in different regions of the liver (Fig. 1b). The hepatocytes in region 1 of acini, the periportal region, exhibited severe vacuolation which varied from minute homogeneously distributed vacuoles to large perinuclear vacuoles (Fig. 1c). The endothelial lining of the central veins and sinusoids were disrupted. The hepatic cord's arrangement was disorganized and several hepatocytes were separated from the cords indicating loss of junction complexes between the adjacent cells. The margins of the hepatocytes were wavy and showed blabbing and probable potential leakage of the material from the cells (Fig. 2a). This is also supposed to distort the integrity of bile canaliculi. The Kupffer cell hyperplasia was noticeable particularly in the zone 3 of acinus, the pericentral region as well as along the highly vacuolated hepatocytes in region 1 of acinus. Higher magnification observations showed that the hepatocytes were swollen and fluffy. The disorganization of hepatic cord arrangement is suggestive of damage to the connective tissue stroma. Following low dose HCM administration the effects were quite similar but comparatively less than the high dose treatment group. The swelling of hepatocytes was prominent but the vacuolation was not as conspicuous. In the recovery groups on day 120, following high dose treatment the hepatocyte swelling was seen almost in the entire lobule with prominence in the zone 1 (periportal region) of the hepatic acinus. Interestingly, several binucleate and polyploid cells were observed both in the low and high dose groups (Fig. 2b). The cytoplasmic dissolution, hepatocyte membrane damage and leakage of cytoplasmic material into the space of Disse as well as within the sinusoid were still prominent on day 120 (Fig. 2c). The extent of variations in the vacuolation in different acinar regions is clearly seen in figures 2 d-f.

DISCUSSION

The present study was aimed to investigate the hepatotoxic effects of low dose exposure of HCM. Animals treated with daily dose of HCM for sixty days induced elevation in liver biomarker enzymes which are considered indicative criteria for damage to hepatocytes [25]. All these liver enzymes, in small amounts, are usually found in blood circulation because of hepatic growth and repair. As a liver specific enzyme, SGPT significantly elevate only in condition of hepatocyte damage, liver parenchyma alterations or in hepatobiliary disease. The available reports on chemical toxicity suggested that increase in activity of liver biomarker enzymes in serum could be due to possible leakage of these enzymes across damaged hepatocyte plasma membrane [26]. In present study, the histoarchitecture of liver in HCM treated groups clearly demonstrated the damage to hepatocyte membrane. Many toxicants either individually or in mixture induced elevation in plasma SGOT, SGPT, GGT and LDH that resulted from leakage of enzymes from damaged tissues of liver [27]. Consequently, elevated levels of biomarker enzymes observed in the current study in response to HCM treatment were common signs of impaired structure and function of liver. The liver cells play an important role in the synthesis and secretion of alkaline phosphatase (ALP) and its location is in the sinusoidal and bile canalicular membranes [28]. The increment in serum ALP activity caused by sixty day HCM treatment again suggests the damaged condition of hepatocytes. Nevertheless, the activity of SGPT and GGT were reduced in serum after withdrawal of HCM treatment until 120 days in both of the recovery groups when compared to sixty day HCM treated groups towards the controls level. The toxicity induced by HCM treatment was lowered during recovery period but the cellular injuries persisted as indicated by higher level of SGOT, ALP and LDH in the serum of recovery groups and the histological observations.

Lipid is an integral part of cell membrane. It is also a source for biosynthesis of several hormones and important for other cellular functions. Lipoproteins enable the transport of cholesterol within the blood stream. Therefore, the measurement of serum cholesterol levels is considered as valuable indicator of lipid metabolism disruption. Results showed that HCM exposure altered the hepatic cellular function in the treated rats by inducing significant increase in the serum TG and cholesterol levels. However, the rise in TG level in HCM exposed group may be due to inhibitory effect of the mixture on the activity of lipoprotein lipase in blood vessel which breaks up the TG [29]. LDL and HDL tend to transport cholesterol into the artery wall and away from the arteries back to the liver, respectively. Several studies have shown that higher levels of LDL promote health problems and cardiac disease [30]. In histological preparations, the extensive vacuolation in the hepatocytes actually suggest lipid accumulation, thus corroborate with the biochemical findings in present and earlier studies [31,32].

Succinate dehydrogenase (SDH) is a key enzyme in the mitochondrial Krebs cycle. Therefore, reduction in SDH activity clearly indicates reduction in aerobic metabolism, which might be the result of reduction of oxygen transport to the tissues. Alterations in the activities of marker enzymes viz., alkaline phosphatase (ALP) and acid phosphatase (ACP) reflect the suppression of liver function [33].

Various studies have reported the effect of toxicants, either individually or in combination, on the oxidative stress in rats [34,35]. It has been linked with enhanced generation of reactive species of oxygen and nitrogen [36]. The reactive oxygen species are very well known to counteract by an interacting network of enzymatic and non enzymatic antioxidant system, thus preventing the cells from oxidative damage [37]. The significant elevated level of LPO in sixty days HCM treated groups indicate generation of oxidative stress.

Oxidative stress/process generated superoxide radical is first converted to hydrogen peroxide and further reduced to give water molecule. In this detoxification pathway superoxide dismutase catalyse the first step and Glutathione peroxidase the next one, where glutathione and catalase help removing hydrogen peroxide. Glutathione-S-transferase (GST) activity detoxifies endogenous compounds such as peroxidised lipid and breakdown product of xenobiotics and may also bind to many toxicants directly and function as transport protein [38-40]. GST also catalyses the conjugation of reduced glutathione to wide variety of substrates and thus the enzyme activity is closely associated with oxidative stress. Ascorbic acid, one of the important antioxidants, reacts with oxidants of the ROS such as hydroxyl radical. The cellular macromolecules are prevented from lipid peroxidation and DNA damage by enzymatic and non enzymatic antioxidant via scavenging the generated ROS. The administration of HCM resulted in the depletion of enzymatic antioxidant (SOD, GPx, CAT and GST) and non enzymatic antioxidant (GSH and ascorbic acid). The depletion in antioxidant enzymes could be attributed to the exhaustive mobilization of these antioxidants to scavenge the ROS associated with HCM hepatocellular toxicity. This decrease of enzymatic antioxidant activity in sixty day treated HCM groups further may result in the deficiency of essential antioxidants to protect the cell from the attack of ROS. The non-enzymatic antioxidant system complements the activity of enzymatic antioxidant system in protecting cells from oxidative stress. There was a significant decrease in the concentration of GSH and ascorbic acid following HCM treatment for 60 days at both the doses. Again, the decrease in concentration of these non-enzymatic antioxidants could be attributed to exhaustion in scavenging ROS. Though the recovery from HCM treatment has no significant effect on GSH and ascorbic acid levels when compared with control but withdrawal groups still show the decrement in these two antioxidants concentrations. The withdrawal from HCM attenuated decreased activity of enzymatic antioxidants (SOD and GPX) and a subsequent recovery towards normalization observed. The withdrawal of treatment, as reported, helped in partial recovery in all the parameters, whereas no changes were observed in the CAT and GST activity. It is interesting to note that the histological preparations distinctly showed that the recovery was partial only. The presence of binucleate cells and polyploid nuclei are indicative of active growth and rapid nuclear divisions for repair and regeneration. However, the metabolic potentials and structure-function correlates like the membrane integrity and leakages across the membrane are sufficient indicators of persistent functional impairment

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