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EFFECT OF COMBINED EXPOSURE TO LEAD AND ETHANOL ON SOME BIOCHEMICAL INDICES IN THE RAT

SWARAN J. S. FLORA* and SUSHIL K. TANDON†
Industrial Toxicology Research Centre, Lucknow-226001, U.P., India

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Abstract—We investigated the effect of daily oral administration to young rats of lead (10 mg/kg) and ethanol (10%, v/v, in drinking water), either alone or in combination, for 8 weeks on the uptake of lead in tissues, brain biogenic amines, hepatic alcohol dehydrogenase and cytosolic and mitochondrial aldehyde dehydrogenase and some selected lead-sensitive variables. Lead given in combination with ethanol produced more pronounced inhibition in the activities of hepatic glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) as compared to lead alone treatment. Simultaneous exposure to lead and ethanol produced a greater depression of dopamine (DA) and 5-hydroxytryptamine (5-HT) levels in the whole brain of rats, compared to rats treated with lead alone. The concentrations of lead in blood, liver and brain were significantly higher in rats exposed simultaneously to lead and ethanol. Though ethanol treatment alone inhibited the activities of hepatic alcohol dehydrogenase and cytosolic and mitochondrial aldehyde dehydrogenase, no effect of lead treatment alone on these variables was observed. The results suggested that animals exposed to ethanol and lead are more vulnerable to the neurologic and hepatotoxic effects and the systemic toxicity of lead.

Lead is a known neurotoxic and hepatotoxic heavy metal [1–4]. Susceptibility to lead intoxication is influenced by a number of physiological and environmental factors [5]. Synergism or antagonism may occur between lead and nutritional factors. A diet low in protein, minerals or certain vitamins may not only enhance the circulating level of lead but may also reduce the general health status of humans and, hence, increase vulnerability to lead intoxication.

The general influence of ethanol on the metabolism of foreign compounds in the body is well known. It has been reported that ethanol enhances the carcinogenicity, mutagenicity [6, 7] and hepatotoxicity [8, 9] of various chemicals. Alcoholic persons are reported to be more susceptible to the toxic effects of lead [10]. Compared to controls matched for time of employment and degree of industrial lead exposure, the workers showing evidence of lead poisoning consumed substantially more ethanol [10]. Alcohol is reported to increase the absorption of lead in the body [11, 12]. Lead poisoning is also associated with consumption of illicit moonshine whiskey. However, Mahaffey *et al.* [13] suggested that the clinically suspected synergism between alcohol consumption and lead poisoning sometimes observed among industrial workers is more likely due to nutritional factors than mutual enhancement of the closely related cellular effects of these two toxins.

There are still relatively few studies available on the effects of ethanol on lead-induced biochemical changes. The present study was undertaken, therefore, to examine the effect of ingested ethanol on the neurotoxic and hepatotoxic effects of lead and on some other lead-sensitive biochemical variables in rats. The activities of some major enzymes involved in hepatic ethanol metabolism were also investigated for possible alterations by lead.

MATERIALS AND METHODS

Male rats (approximately 60 g) of the MRC strain inbred in the Industrial Toxicology Research Center colony were quarantined and, after adaptation to the animal quarters for 2 weeks, they were randomly distributed into four groups of six rats each. Each group was assigned to one of the following treatments for 8 weeks: Group I—no treatment (normal control), Group II—ethanol (10%, v/v) in drinking water, Group III—lead as lead acetate (10 mg/kg, orally, single dose daily), and Group IV—lead as lead acetate (10 mg/kg, orally, single dose, daily) plus ethanol (10%) in drinking water.

All animals received standard chow diet (Hindustan Lever Ltd., India) *at lib*. After 8 weeks of lead and/or ethanol exposure, each animal was placed in separate metabolic cages for 24 hr. Urine was collected for the estimation of δ-aminolevulinic acid (δ-ALA). All rats were then stunned, decapitated, and exsanguinated. Blood was collected in heparinized vials, and major organs were immediately removed, weighed and processed for analytical determination.

Livers were removed immediately and washed in ice-cold 0.25 M sucrose. These livers were trimmed, blotted dry, and weighed. Livers for reduced glutathione determination were obtained from animals 8 hr after ethanol treatment and were washed, trimmed, and frozen at −75°.

* Present address: Utah State University, Toxicology Program, Logan, UT 84322-4620, U.S.A.
† To whom reprint requests should be addressed.

Livers for enzymatic analyses were obtained 4 hr after ethanol treatment. These livers were homogenized with a Teflon pestle in 4 vol. of ice-cold 0.25 M sucrose. The homogenate was centrifuged at 4° at 500 *g* for 15 min. The crude nuclear pellet was resuspended in a volume of 0.25 M sucrose equal to that used in the original homogenate. This resuspension was centrifuged again at 500 *g* for 10 min. The resulting supernatant fraction was combined with the supernatant fraction of the first centrifugation and recentrifuged at 7000 *g* for 10 min. The resulting mitochondrial pellet was washed two times with a volume of 0.25 M sucrose equal to one-fourth the original homogenate volume. The original post-mitochondrial supernatant fraction was centrifuged at 100,000 *g* for 1 hr for preparation of the cytosolic fraction. The washed mitochondrial pellet was resuspended in 5 mM phosphate buffer, pH 7.4, at a volume equal to twice the original liver weight. Sodium deoxycholate was added to obtain 2 mg/g of the original liver weight. The lysed mitochondria were centrifuged at 27,000 *g* for 20 min. The supernatant fraction of this preparation was employed for mitochondrial enzymatic analyses.

Analytical procedures. Mitochondrial aldehyde dehydrogenase (ALDH) activity was determined spectrophotometrically by measuring the production of NADH as described by Petersen *et al.* [14] except that the reaction was performed at pH 7.4. The reaction mixture contained 5 mM propionaldehyde and 1.0 mM NAD^+ (Sigma) in 60 mM phosphate buffer, pH 7.4. All enzymatic analyses were performed at 32° using a Beckman 25 recording spectrophotometer. Cytosolic aldehyde dehydrogenase activity was assayed using post-microsomal supernatant fraction. Cytosolic ALDH activity was determined by following the production of NADH as described by Petersen *et al.* [14] except that the reaction pH was 7.4 instead of 8.8. Each reaction mixture contained 5.0 mM propionaldehyde, 1.0 mM NAD^+, 1.0 mM pyrazole (to inhibit enzymatic oxidation of NADH and subsequent reduction of propionaldehyde to propionol) in 60 mM phosphate buffer, pH 7.4.

Alcohol dehydrogenase activity was assayed by using a modification of the method of Bonnichsen and Brink [15]. The reaction mixture contained 16 mM ethanol, 1.6 mM NAD^+, and 30 mM semicarbazide in 60 mM phosphate buffer, pH 7.4. Semicarbazide was added to trap the reaction product, acetaldehyde, so that cytosolic ALDH-mediated production of NADH would not occur.

Mitochondrial and cytosolic protein content was determined using the biuret method as described by Gornall *et al.* [16]. The activities of glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) in liver were measured according to the procedure of Reitman and Frankel [17] by estimating their hydrazone at 510 nm.

Reduced hepatic glutathione (GSH) content was determined by the *o*-phthaldehyde fluorometric method of Hissin and Hilf [18].

All subsequent operations after removing the brain were performed at 4°. Brain tissue from each rat was divided into two halves by median sagittal section; one half was used for the estimation of biogenic amines and the other half for lead contents. The biogenic amines were extracted and estimated according to the procedure of Sadavongvivad [19]. Protein was estimated by the method of Lowry *et al.* [20].

The activity of δ-aminolevulinic acid dehydratase (δ-ALAD) and the hemoglobin contents in blood were estimated by the method of Berlin and Schaller [21] and Clegg and King [22] respectively. Urinary excretion of δ-ALA was measured by the method of Davis *et al.* [23].

Estimation of lead. Lead was estimated in blood [24], kidneys, liver and brain [25] by atomic absorption spectrophotometry (Perkin–Elmer model 5000).

Statistical analyses. Data were subjected to a one-way analysis of variance test. Differences between control and treatment means were determined by Dunnett's test [26].

RESULTS

The treatment with lead or ethanol did not cause any significant change in body weight gain of animals compared to the normal control. However, animals given lead and ethanol together showed significant weight loss compared to animals treated with lead or ethanol alone (data not shown).

Table 1 shows that the administration of lead alone inhibited the activity of δ-ALAD and the hemoglobin contents in blood while significantly elevating the level of δ-ALA in urine. Ethanol administration alone also inhibited the activity of δ-ALAD in blood but no alteration occurred in either blood Hb or

Table 1. Effects of ethanol, lead and their combined exposure on some blood and urine variables in rats

Treatment	Blood δ-ALAD (μmoles δ-ALA/ min/l erythrocytes)	Blood Hb (g/100 ml)	Urine δ-ALA (mg/100 ml)
Normal control	5.87 ± 0.53*	12.42 ± 0.21*	0.11 ± 0.01*
Ethanol	4.17 ± 0.09*	12.41 ± 0.33*	0.13 ± 0.01*
Lead	3.12 ± 0.37†	10.69 ± 0.36†	0.71 ± 0.02†
Lead + ethanol	4.43 ± 0.47*	10.87 ± 0.34†	0.81 ± 0.06†

Values are mean ± SE; N = 6.

*† Values with matching superscripts in each column are not different at the 5% level of significance.

Table 2. Effects of ethanol, lead and their combined exposure on hepatic alcohol dehydrogenase (ADH) and cytosolic and mitochondrial aldehyde dehydrogenase (ALDH) activities in rats

Treatment	ADH	Cytosolic ALDH	Mitochondrial ALDH
	(nmoles NADH produced/min/mg protein)		
Normal control	21.88 ± 0.87*	23.52 ± 0.62*	23.31 ± 0.79*
Ethanol	14.26 ± 2.13†	14.66 ± 0.94†	16.37 ± 0.87†
Lead	20.51 ± 1.21*	24.17 ± 0.85*	21.53 ± 0.99*
Lead + ethanol	13.75 ± 0.76†	10.72 ± 0.90‡	12.53 ± 0.63‡

Values are mean ± SE; N = 6.
*–‡ Means with matching symbol notations within the same column are not different at the 5% level of significance.

urinary δ-ALA levels. Animals given both ethanol and lead showed no marked inhibition in blood δ-ALAD activity compared to lead alone treated animals.

Table 2 shows the activities of some major enzymes involved in hepatic ethanol metabolism. The specific activities of hepatic cytosolic and mitochondrial ALDH were decreased in ethanol alone treated rats. The specific activity of hepatic ADH also decreased significantly in ethanol treated rats. The magnitude of inhibition in the activities of cytosolic and mitochondrial ALDH and hepatic ADH was greater in animals treated with lead and ethanol compared to ethanol or lead alone treated rats.

The effects of ethanol, lead, or lead + ethanol treatment on the hepatic transaminase activities and glutathione contents are depicted in Table 3. Activities of hepatic GOT and GPT decreased significantly in ethanol alone treated rats, whereas lead alone treatment decreased the activity of hepatic GPT and enhanced the level of glutathione. A combined exposure to lead and ethanol led to a significant inhibition of hepatic GOT and GPT activities and a reduced level of glutathione contents compared to lead alone treated rats.

Results presented in Table 4 indicate that the administration of lead alone decreased the level of dopamine (DA) and 5-hydroxytryptamine (5-HT) and significantly increased the level of norepinephrine (NE). No significant differences in the levels of DA, NE or 5-HT were observed due to ethanol alone treatment, although animals coadministered with lead and ethanol showed a more pronounced decrease in the levels of DA and 5-HT and a significantly increased level of NE in whole brain.

Table 3. Effects of ethanol, lead or their combined exposure on hepatic transaminase activities and glutathione concentration in rats

Treatment	GOT	GPT	GSH
	(nmoles hydrazone formed/min/mg protein)		(μmoles/g fresh tissue)
Normal control	30.81 ± 2.53*	30.55 ± 1.15*	6.64 ± 0.74*
Ethanol	25.36 ± 0.84*	25.23 ± 1.29†	8.18 ± 0.87*
Lead	26.51 ± 2.13*	26.80 ± 1.00†	11.03 ± 0.76*
Lead + ethanol	17.80 ± 0.77†	18.43 ± 0.76‡	9.79 ± 0.41*

Values are mean ± SE; N = 6.
*–‡ Means with matching symbol notations in each column are not different at the 5% level of significance.

Table 4. Effects of ethanol, lead and their combined exposure on some brain biogenic amine levels in rats

Treatment	DA	NE	5-HT
		(μg/g fresh tissue)	
Control	0.36 ± 0.01*	0.44 ± 0.01*	0.54 ± 0.03*
Ethanol	0.34 ± 0.01*	0.47 ± 0.02*	0.49 ± 0.03*
Lead	0.30 ± 0.01†	0.52 ± 0.02*	0.40 ± 0.02†
Lead + ethanol	0.25 ± 0.01†	0.49 ± 0.02*	0.34 ± 0.01‡

Values are mean ± SE; N = 6.
*–‡ Means with matching symbol notations within the same column are not different at the 5% level of significance.

Table 5. Effects of ethanol, lead, and their combined exposure on the blood and tissue concentrations of lead in rats

Treatment	Blood (μg/100 ml)	Liver (μg/g)	Kidney (μg/g)	Brain (μg/g)
Control	5.23 ± 1.05*	1.52 ± 0.07*	2.41 ± 0.33*	0.48 ± 0.08*
Ethanol	5.53 ± 0.37*	1.56 ± 0.25*	2.53 ± 0.45*	0.53 ± 0.05*
Lead	78.23 ± 16.86†	12.13 ± 0.94†	12.94 ± 1.10†	3.54 ± 0.22†
Lead + ethanol	129.75 ± 10.17‡	16.16 ± 0.40‡	13.47 ± 0.96†	5.45 ± 0.36‡

Mean ± SE for six observations.
*–‡ Values marked by a different symbol are significantly different from each other at the 5% level of significance.

Data in Table 5 indicate that the concentration of lead increased significantly after its administration in liver, kidney, brain and blood. A simultaneous exposure to lead and ethanol led to a significantly higher concentration of lead in liver, brain and blood compared to lead alone treated rats.

DISCUSSION

Lead has a pronounced neurotoxicity in both humans and animals; chronic exposure to low levels of lead may induce mental retardation and hyperactivity in humans [27, 28]. Central nervous system toxicity due to excessive lead absorption occurs at lower levels of exposure than dysfunction of the peripheral nervous system [29]. Chronic exposure of young rodents to low levels of lead has been reported to affect the CNS as shown by alterations in behavior and neurotransmitter systems [30]. Motor activity is the behavior most often reported to change following lead exposure [31], although hyperactivity [32] and normal activity have also been reported [33]. The involvement of brain catecholamines in lead-induced hyperactivity has been investigated by several workers. Catecholamine, especially dopamine, appears to be involved in the central regulation of motor activity [34]. Some studies have suggested that lead treatment produces small but possibly significant alterations in steady-state levels of dopamine in lead treated animals [35], whereas others report no changes with lead treatment [36]. Jason and Kellogg [37] utilized gastric intubation to produce brain lead levels in the 0.6 μg/g range and found a 25% decrease in striatal DA levels at postnatal day 15 which persisted to day 35 even though the brain lead level decreased by over half. An increase in the NE level in whole brain was reported by Satija *et al.* [38]. Findings concerning 5-HT levels are also contradictory. Silbergeld and Goldberg [39] found no effect on 5-HT uptake. There are relatively few studies available on the long-term effects of ethanol on the brain catecholamine system. Using mice, Rawat [40] found that the chronic administration of ethanol in a liquid diet for 4 weeks resulted in a decrease in brain NE contents but, after withdrawal of ethanol for 1 day, the level of NE returned to normal.

Intermediary metabolism of ethanol has been widely studied, and it is generally agreed that L-ADH is the principal enzyme involved in the metabolism of ethanol [41]. There is a controversy over whether ethanol consumption affects activities of hepatic ADH, with increases [42] or no changes [43] or even decreases reported. Some who found a moderate initial increase subsequently observed a return to normal or a decrease after prolonged ethanol feeding [44]. A decrease in the normal level of ADH in our study could be explained by the fact that, at later stages of alcoholism, the damaged liver cell has a decreased ability to form ADH. This was also observed by Dajani *et al.* [44] but only after 24 weeks of alcohol administration. Figueroa and Klotz [45] also reported a decrease in the ADH levels in liver taken by biopsy from human alcoholics with cirrhosis. The inhibition of hepatic cytosolic and mitochondrial ALDH and inhibition of hepatic GOT and GPT by ethanol suggest the production of liver necrosis by ethanol [46]. Administration of lead alone also inhibited the hepatic activity of GPT showing hepatotoxicity by lead [4] but no change in hepatic ADH or ALDH was observed. The most marked potentiation of hepatotoxicity was observed in rats simultaneously exposed to lead and ethanol as compared to either lead or ethanol. The potentiating effects of ethanol on the hepatotoxic effects of several chemicals are well known [8, 9].

A lead-induced increased level of glutathione (GSH) in liver can be explained by the fact that the lead might be binding with GSH in order to overcome the toxicity [47]. It is a compensatory mechanism. The formation of GSH conjugation with lead remains a possible explanation [47]. One of the most sensitive sites for lead effects is the intramitochondrial enzyme, δ-aminolevulinic acid dehydratase, a step in the biochemical synthesis of heme. Increased urinary excretion of δ-ALA, a metabolic intermediate, reflects organ lead inhibition of heme synthesis and inhibition of δ-ALAD activity in blood [13], though both lead and ethanol are reported to inhibit δ-ALAD activity, but together they act in opposite directions and inhibition of δ-ALAD does not occur [13]. Ethanol treatment enhanced the concentration of lead in blood, liver and brain. This effect may explain possible enhancement of lead toxicity as an effect to increase internal dose. Thus, the finding of increased δ-ALAD activity with combined lead + ethanol exposure may result solely from the increased amount of lead in blood under conditions of simultaneous exposure to ethanol.

The results reported here indicate that rats subjected to combined exposure of lead and ethanol

showed more vulnerability to lead toxicity and more pronounced hepatotoxic and neurotoxic effects, though it is not clear about the basis for the apparent synergism between alcohol and lead, particularly at the molecular level. Goyer and Mahaffey [48] reported that, if one compares the cellular pathology of lead and ethanol, similarities can be observed. Each alone can produce mitochondrial injuries. Cramer [10] reported that *in vitro* studies of mitochondria from ethanol treated rats show decreased oxidative capacity and increased membrane permeability. It is also suspected that ethanol may cause various nutritional deficiencies, e.g. calcium, protein and vitamin which enhance the toxicity of lead [48].

The interactive effects between lead and ethanol appear to be on variables that are primarily related to lead toxicity as well as on those more characteristic of ethanol toxicity. Thus, it can be concluded that ethanol exposure may potentiate the toxic effects of lead as well as vice versa. It was not possible to single out any particular factor that caused increased lead toxicity but ethanol seems to be effective in breeching the blood–brain and blood–gastric barrier, thereby enhancing the uptake of lead in blood and other organs.

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