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Enhancement of Lindane-induced Liver Oxidative Stress and Hepatotoxicity by Thyroid Hormone is Reduced by Gadolinium Chloride

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The role of Kupffer cells in the hepatocellular injury and oxidative stress induced by lindane (20 mg/kg; 24 h) in hyperthyroid rats (daily doses of 0.1 mg L-3,3′,5-triiodothyronine (T_3)/kg for three consecutive days) was assessed by the simultaneous administration of gadolinium chloride ($GdCl_3$; 2 doses of 10 mg/kg on alternate days). Hyperthyroid animals treated with lindane exhibit enhanced liver microsomal superoxide radical ($O_2^{\cdot -}$) production and NADPH cytochrome c reductase activity, with lower levels of cytochrome P450, superoxide dismutase (SOD) and catalase activity, and glutathione (GSH) content over control values. These changes are paralleled by a substantial increase in the lipid peroxidation potential of the liver and in the $O_2^{\cdot -}$ generation/SOD activity ratio, thus evidencing a higher oxidative stress status that correlates with the development of liver injury characterized by neutrophil infiltration and necrosis. Kupffer cell inactivation by $GdCl_3$ suppresses liver injury in lindane/T_3-treated rats with normalization of altered oxidative stress-related parameters, excepting the reduction in the content of GSH and in catalase activity. It is concluded that lindane hepatotoxicity in hyperthyroid state, that comprises an enhancement in the oxidative stress status of the liver, is largely dependent on Kupffer cell function, which may involve generation of mediators leading to pro-oxidant and inflammatory processes.

Keywords: Lindane; Thyroid hormone; Gadolinium chloride; Oxidative stress; Liver injury; Kupffer cells

INTRODUCTION

The oxidative stress status of the liver can be enhanced by molecular mechanisms related to the biotransformation of xenobiotics such as lindane,[1] the catalytic action of transition metals such as iron and copper in reactions leading to free radical generation,[2] or to acceleration of energy metabolism by thyroid calorigenesis.[3] Biotransformation of lindane by microsomal cytochrome P450 system is associated with an early depletion of glutathione (GSH), due to conjugation with electrophilic metabolites for elimination. This is followed by induction of cytochrome P450, with the consequent enhancement of superoxide radical formation and lipid peroxidation[1] and protein oxidation[4] indicators. In addition, antioxidant enzyme functioning is also compromised thus increasing the oxidative stress status of the liver, a change that is closely related and coincides with the onset and progression of morphological lesions.[1]

Recently, data have been reported to support the contention that concurrence of processes related to oxidative stress elicited by different experimental conditions either induces or potentiates liver injury.

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This response involves additive or synergistic increases in the oxidative stress status of the liver as shown in hyperthyroid rats subjected to lindane intoxication,[5,6] ischemia–reperfusion,[7,8] or iron overload.[9] Moreover, lindane treatment sensitizes the liver to the damaging effects of iron overload by providing an added increase to the oxidative stress status of the tissue.[10] In agreement with these studies, thyroid hormone-induced sensitization to hepatotoxicity has also been reported for halothane, isoflurane and enflurane, carbon tetrachloride, thioacetamide, 1,1-dichloroethylene, and chloroform.[3] Furthermore, liver injury associated with thioacetamide intoxication, cold organ storage in liver transplantation, or that produced by the exposure of rats chronically treated with ethanol to low O_2 tensions is substantially diminished by hypothyroid state elicited by either methimazole and propylthiouracil administration or thyroidectomy.[3] These observations suggest that thyroid status is an important factor in the development and progression of various types of liver diseases or in their prevention.

Kupffer cells, the resident macrophages of the liver, are involved in immunomodulation, phagocytosis, and biochemical attack, through production and release of biologically active products such as proteases, free radicals, and peptide mediators.[11] These active products of stimulated Kupffer cells underlie their bactericidal and tumoricidal effects; however, harmful responses such as cytotoxicity and inflammation are achieved when liver macrophage functioning is exacerbated.[11,12] In view of these observations and considering that low doses of lindane[13] and hyperthyroid state[14] significantly enhance the respiratory burst activity of Kupffer cells representing an alternate mechanism contributing to oxidative stress in the liver, the aim of this study was to investigate the involvement of liver macrophages in the hepatotoxicity of lindane in hyperthyroid rats. For this purpose, rats made hyperthyroid by L-3,3,5-triiodothyronine (T_3) administration were subjected to a low dose of lindane (20 mg/kg), in the absence or presence of gadolinium chloride ($GdCl_3$) treatment, a rare earth metal known to selectively induced Kupffer cell depletion.[15] Parameters related to oxidative stress in the liver were determined in relation to the morphological characteristics of the tissue obtained by light microscopy assessment.

MATERIALS AND METHODS

Animals and Treatments

Male Sprague–Dawley rats weighing about 250 g received daily i.p. injections of 0.1 mg of T_3/kg body weight or equivalent volumes of 0.1N NaOH (T_3 diluent) for three consecutive days. Additionally, animals received two doses of 10 mg of $GdCl_3$/kg i.v. or equivalent volumes of 0.9% (w/v) NaCl, on alternate days, starting from the first day of hormone treatment. On the third day of hormone treatment, animals were also given either 20 mg of lindane/kg or equivalent volumes of corn oil, i.p. The combination of these treatments resulted in eight experimental groups: (a) control rats, (b) lindane-treated rats, (c) hyperthyroid rats, (d) lindane-treated hyperthyroid rats, (e) $GdCl_3$-treated rats, (f) $GdCl_3$-lindane-treated rats, (g) $GdCl_3$-treated hyperthyroid rats, and (h) $GdCl_3$-lindane-treated hyperthyroid rats. Determinations were carried out 24 h after the last treatment. The rectal temperature of the animals was taken before sacrifice. All the animals used, received humane care according to the guidelines outlined in the Guide for the Care and Use of Laboratory Animals by the National Academy of Sciences (National Institutes of Health publication 86–23).

Biochemical Parameters

The animals were euthanized by cervical dislocation and the abdominal cavities were open. Liver samples were taken and homogenized in 0.5N $HClO_4$ for determination of total glutathione (GSH) content.[16] Livers were perfused *in situ* with ice-cold 0.9% (w/v) NaCl, homogenized (25%, w/v) in 140 mM KCl and 10 mM potassium phosphate buffer pH 7.0, centrifuged at 900*g* for 20 min at 4°C, and the supernatants were used for lipid peroxidation potential determination by means of thiobarbituric acid reactants (TBARs) production.[17] The post-microsomal supernatants were used for the assessment of superoxide dismutase (SOD)[18] and catalase[19] activities. Part of the microsomal pellet was resuspended in 0.1 M citrate buffer containing 0.1 M KCl, 30% (v/v) glycerol, and 1 mM DTT, pH 7.0, for cytochrome P450 assessment.[20] The rest of the pellet was resuspended in 140 mm KCl and 10 mM potassium phosphate buffer with pH 7.0 for determination of NADPH cytochrome c reductase activity[21] and superoxide anion production ($O_2^{\cdot -}$).[22] Protein was assessed as in Lowry *et al.*[23]

Histological Studies

Liver samples from all experimental groups were fixed in 10% buffered formaldehyde, paraffin embedded, and stained with hematoxylin-eosin.

Statistical Analysis

The statistical significance of differences among multiple groups was assessed by the nonparametric

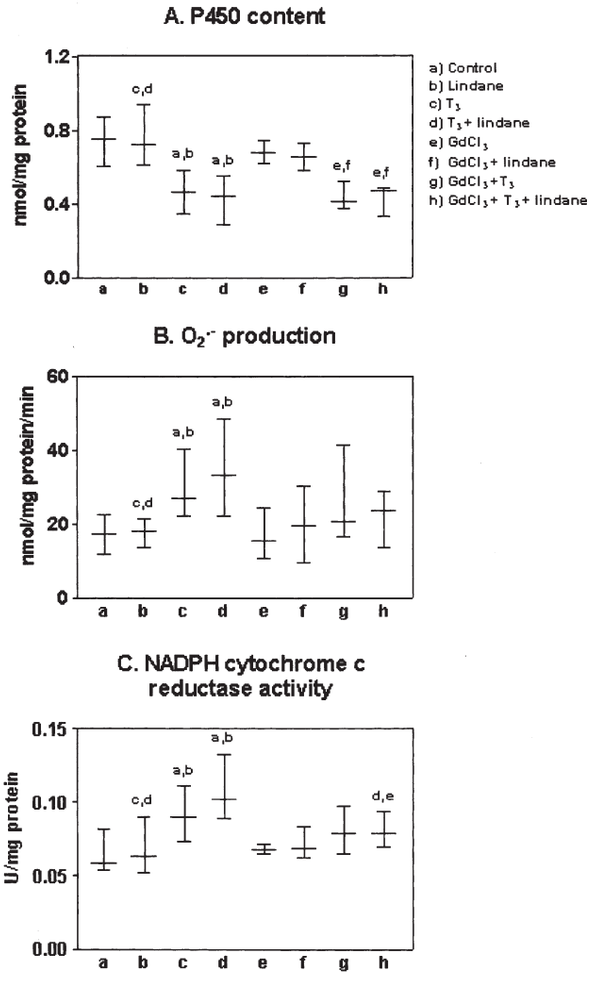

FIGURE 1 Liver microsomal cytochrome P450 content (A), superoxide radical ($O_2^{\cdot-}$) generation (B), and NADPH cytochrome c reductase activity (C) in control rats and animals subjected to either lindane, T_3, and/or gadolinium chloride ($GdCl_3$) administration as described in "Materials and Methods" Section. Values shown are medians with the respective minimal and maximal values for 4–12 animals per experimental group. The statistical significance of differences among multiple groups was detected by Kruskall–Wallis test followed by Mann–Whitney test and is shown by the letters identifying each group [$^a p < 0.05$ versus control; $^b p < 0.05$ versus lindane-treated rats; $^c p < 0.05$ versus hyperthyroid animals; $^d p < 0.05$ versus hyperthyroid rats treated with lindane; $^e p < 0.05$ versus $GdCl_3$-treated rats; $^f p < 0.05$ versus $GdCl_3$ and lindane-treated animals].

test of Kruskall–Wallis followed by Mann–Whitney test. Results are medians with the respective minimal and maximal values for the number of separate experiments shown.

RESULTS

Thyroid calorigenesis was evidenced by the significant increases in the rectal temperature of the animals [in means ± SEM and significance analysis done by one-way ANOVA and the Newman–Keuls' test, (a) controls, 36.7 ± 0.2°C ($n = 4$); (b) lindane-treated rats, 37.1 ± 0.1 ($n = 4$); (c) hyperthyroid rats, 37.7 ± 0.1 ($n = 5$) [$p < 0.05$ vs. (a) and (b)]; (d) hyperthyroid rats subjected to lindane treatment, 38.1 ± 0.2 ($n = 4$) [$p < 0.05$ vs. (a) and (b)]. $GdCl_3$ treatment did not interfere with the calorigenic response of T_3 [(e) $GdCl_3$-treated rats, 37.6 ± 0.1 ($n = 5$); (f) $GdCl_3$-treated rats subjected to lindane intoxication, 37.2 ± 0.03 ($n = 4$); (g) $GdCl_3$-treated hyperthyroid rats, 38.3 ± 0.03 ($n = 4$) ($p < 0.05$ vs. (e) and (f); (h) $GdCl_3$-treated rats subjected to T_3 and lindane administration, 38.1 ± 0.1 ($n = 4$) ($p < 0.05$ vs. (e) and (f)].

Hyperthyroid rats showed a decrease in cytochrome P450 content (Fig. 1A), accompanied by an increase in both NADPH cytochrome c reductase activity (Fig. 1B) and microsomal $O_2^{\cdot-}$ production (Fig. 1C). These microsomal parameters were not modified further by lindane administration, however, $GdCl_3$ treatment suppressed the increases in microsomal $O_2^{\cdot-}$ production and NADPH cytochrome c reductase activity elicited by T_3, without changing the decrease in cytochrome P450 content (Fig. 1). In these conditions, hyperthyroidism led to a decrease in hepatic SOD activity independently of lindane intoxication, which was not observed in hyperthyroid rats subjected to $GdCl_3$ treatment (Fig. 2A). Liver catalase activity was also diminished by T_3 independently of lindane treatment, an effect that was further exacerbated upon $GdCl_3$ administration (Fig. 2B). In addition, hyperthyroidism led to GSH depletion in the liver, a parameter that was further reduced by the combined hormone–lindane administration and was not modified by $GdCl_3$ (Fig. 2C). Both T_3 and lindane enhanced the lipid peroxidation potential of the liver, with a significant additional increase being produced by the combined administration, effects that were not observed upon $GdCl_3$ treatment (Fig. 3A). Interestingly, both hepatic catalase activity and lipid peroxidation potential of animals subjected to $GdCl_3$ administration were lower than those found in control rats (Figs. 2B and 3A). Finally, T_3-induced enhancement in the $O_2^{\cdot-}$ generation/SOD activity ratio was significantly augmented by the combined hormone–lindane treatment, effects that were abolished by treatment with $GdCl_3$ (Fig. 3B).

Histological analysis of liver samples from controls (Fig. 4A) and lindane-treated (Fig. 4B) rats revealed normal morphology. T_3 treatment also resulted in normal parenchymal cell morphology, with significant Kupffer cell hyperplasia. Kupffer cells were morphologically identified in hyperthyroid samples based on their irregular, eosinophilic cytoplasm and intensely basophilic, elongated nucleus (Fig. 4C). Lindane intoxication in hyperthyroid rats led to Kupffer cell hyperplasia, accompanied by polymorphonuclear leukocyte infiltration and granuloma formation (Fig. 4D), in addition to parenchymal alterations which characterize the first

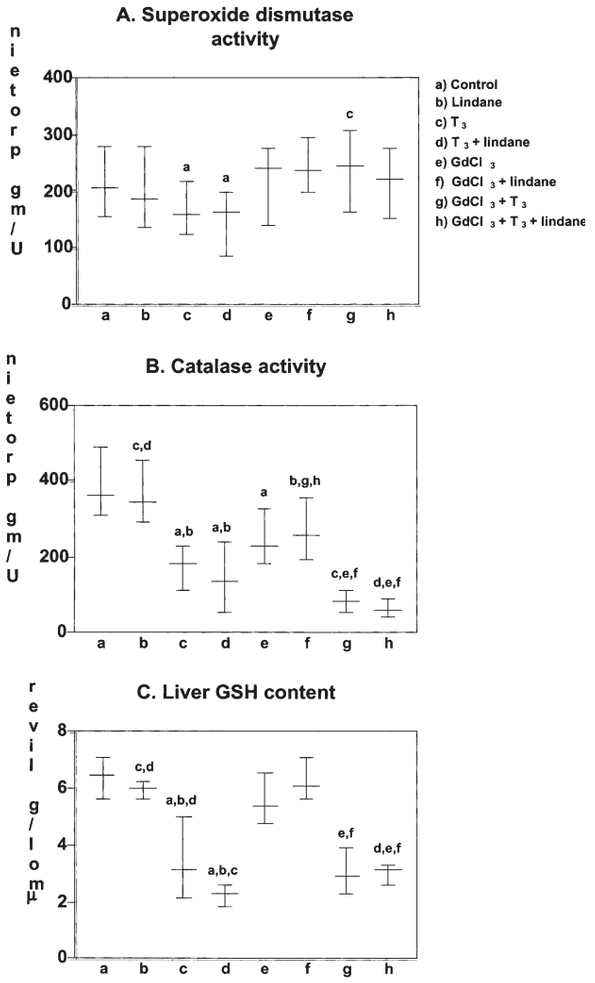

FIGURE 2 Liver superoxide dismutase activity (A), catalase activity (B), and total reduced glutathione (GSH) content (C) in control rats and animals subjected to either lindane, T_3, and/or gadolinium chloride ($GdCl_3$) administration as described in "Materials and Methods" section. Values shown are medians with the respective minimal and maximal values for four to nine animals per experimental groups. The statistical significance of differences among multiple groups was detected by Kruskall–Wallis test followed by Mann–Whitney test and is shown by the letters identifying each group [$^{a}p < 0.05$ versus control; $^{b}p < 0.05$ versus lindane-treated rats; $^{c}p < 0.05$ versus hyperthyroid animals; $^{d}p < 0.05$ versus hyperthyroid rats treated with lindane; $^{e}p < 0.05$ versus $GdCl_3$-treated rats; $^{f}p < 0.05$ versus $GdCl_3$ and lindane-treated animals].

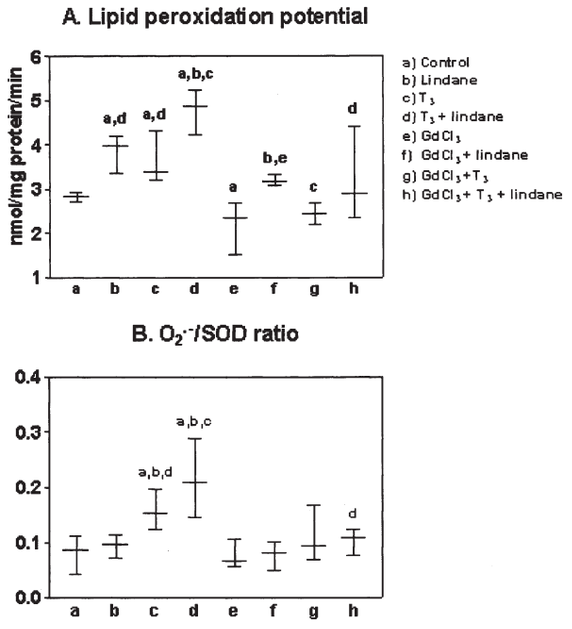

FIGURE 3 Liver lipid peroxidation potential (A) and superoxide radical production/superoxide dismutase activity ($O_2^{\bullet-}$/SOD) ratio (B) in control rats and animals subjected to either lindane, T_3, and/or gadolinium chloride ($GdCl_3$) administration as described in "Materials and Methods" section. Lipid peroxidation potential was assessed by means of production of thiobarbituric acid reactants. Values shown are medians with the respective minimal and maximal values for 4–7 animals per experimental group. The statistical significance of differences among multiple groups was detected by Kruskall–Wallis test followed by Mann–Whitney test and is shown by the letters identifying each group [$^{a}p < 0.05$ versus control; $^{b}p < 0.05$ versus lindane-treated rats; $^{c}p < 0.05$ versus hyperthyroid animals; $^{d}p < 0.05$ versus hyperthyroid rats treated with lindane; $^{e}p < 0.05$ versus $GdCl_3$-treated rats].

signs of cell necrosis, shown as hepatocytes with picnotic nucleus and highly basophilic cytoplasm (Fig. 5). These alterations were not observed in T_3-lindane-treated rats subjected to $GdCl_3$ administration, i.e. Kupffer cell depletor that did not alter liver morphology (Fig. 4, E–H).

DISCUSSION

Data presented in this study support the contention that hyperthyroidism increases the susceptibility of the liver to the toxic effects of lindane.[5,6] This effect may be due to (i) a high free radical activity imposed by thyroid calorigenesis secondary to the increased serum levels of T_3 induced by lindane and (ii) the generation of reactive lindane-derived metabolites coupled to an accelerated biotransformation of the insecticide, suggested by the decreased serum and hepatic levels of lindane in hyperthyroid state.[5] Furthermore, the administration of lindane to hyperthyroid rats elicited significant alterations in microsomal function leading to enhanced $O_2^{\bullet-}$ generation, with decreased activity of the antioxidant enzymes SOD and catalase and GSH depletion, thus exacerbating the oxidative stress status of the liver. Reduction in the content of hepatic lipid-soluble antioxidant vitamins (α-tocopherol, β-carotene, and lycopene) also contributes to this redox unbalance in this experimental model.[6] Consequently, enhancements in the lipid peroxidation potential and in the $O_2^{\bullet-}$/SOD ratio of the liver are observed, with the parallel development of liver injury not found in hyperthyroid rats or in animals subjected to lindane treatment separately.

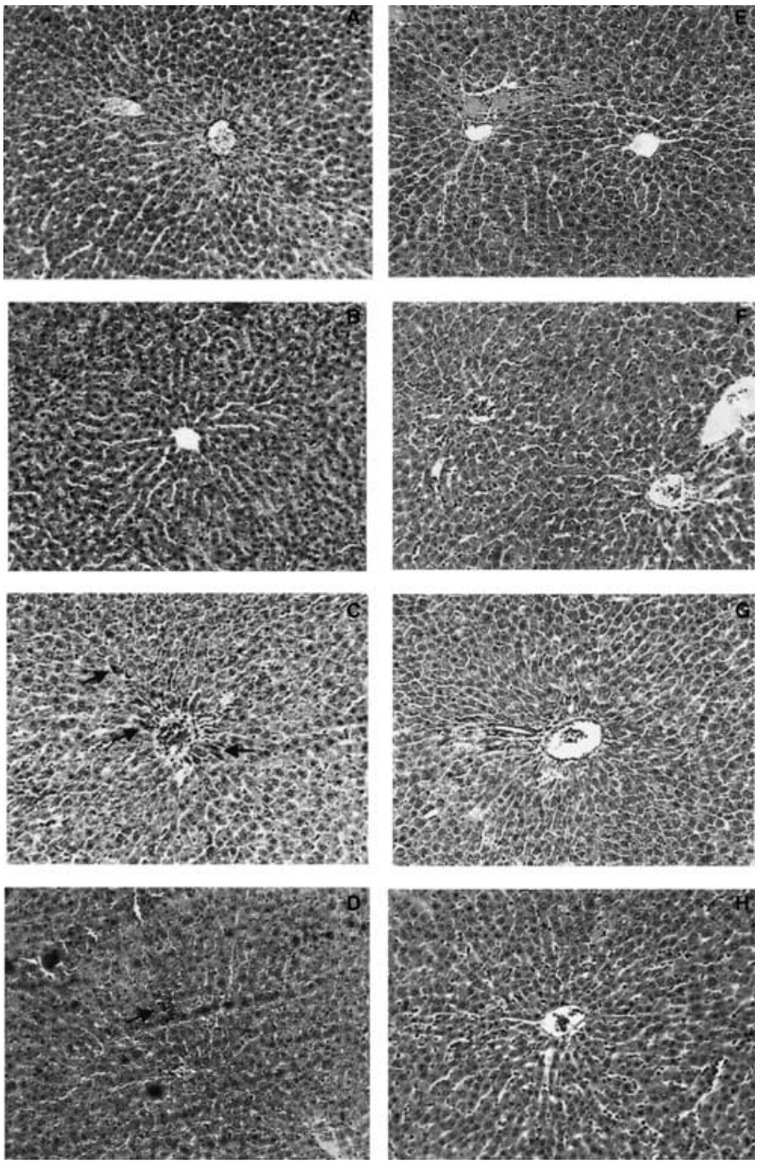

FIGURE 4 Structural characteristics of liver parenchyma in (A) a control rat and in animals subjected to the administration of either (B) lindane, (C) T_3, (D) lindane and T_3, (E) $GdCl_3$, (F) $GdCl_3$ and lindane, (G) $GdCl_3$ and T_3, or $GdCl_3$, lindane, and T_3, as described in "Materials and Methods" section. Livers from animals in groups A, B, E, F, G, and H exhibit normal parenchyma with intact hepatocytes, whereas group C shows Kupffer cell hyperplasia with heavily stained Kupffer cells (arrows) and group D exhibits infiltration of polymorphonuclear leukocytes (arrow) (hematoxylin-eosin, original magnification 100 ×).

The observation that $GdCl_3$ treatment suppresses liver injury in lindane/T_3-treated rats ascribes to Kupffer cells a major pathogenic role, considering that liver macrophages release a variety of cytotoxic mediators upon stimulation.[11] Thus, Kupffer cell depletion by $GdCl_3$ may avoid the oxidative stress component associated with the enhanced respiratory burst activity observed in rats given lindane[13] or T_3,[14] a process known to involve generation of reactive oxygen and nitrogen species.[24] This suggestion is in agreement with the $GdCl_3$-induced normalization of the elevated lipid peroxidation potential and the $O_2^{\cdot -}$/SOD ratio found in lindane/T_3-treated rats. In addition, derangement in the expression of genes encoding for pro- and/or anti-inflammatory cytokines by reactive oxygen species cannot be discarded,[25] as hydrogen peroxide produced by the respiratory burst of macrophages is known to activate transcription factors such as NF-κB.[26] Activation of NF-κB and other nuclear factors by oxidative stress appears to exert a prominent transcriptional control over expression of mediators involved in hepatotoxicity, known to be released by Kupffer cells and other sinusoidal cells.[11] However, further studies are required to verify this proposal. In addition to inhibition of

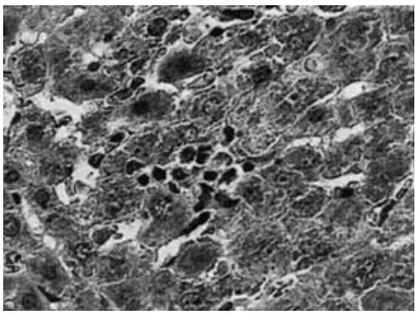

FIGURE 5 Histopathology of rat liver after the combined administration of lindane and T_3 (group D in Fig. 4), showing polymorphonuclear leukocyte infiltration and isolated necrotic hepatocytes (hematoxylin-eosin, original magnification 400 ×).

Kupffer cell function, protection of chemical-induced hepatotoxicity by $GdCl_3$ may involve a decreased cytochrome P450-mediated bioactivation.[27] However, the latter mechanism does not seem to play a significant role in reducing the hepatotoxicity observed in lindane/T_3-treated rats subjected to $GdCl_3$ pretreatment. This contention is supported by the findings that cytochrome P450 content, NADPH cytochrome c reductase activity, and microsomal NADPH-dependent $O_2^{\cdot -}$ production observed in $GdCl_3$-treated rats are comparable to those in control animals. Furthermore, decreased biotransformation of xenobiotics by $GdCl_3$ may be circumscribed to reduction in the content of specific isoforms of cytochrome P450, as shown for CYP2E1.[28]

Collectively, studies reported demonstrate that (i) administration of lindane to hyperthyroid rats elicits a major increase in the oxidative stress status of the liver over those observed after the separate treatments, (ii) this effect is paralleled by development of liver injury, and (iii) Kupffer cells play an important role in determining oxidative stress and the morphological changes described. The conclusions drawn are in line with the observation that the hepatotoxic effect of several drugs is enhanced by hyperthyroid state or blunted by antithyroid therapy,[3] including that of acetaminophen,[29] carbon tetrachloride,[30] or ethanol.[31] These agents are known to promote oxidative stress[32–34] and display hepatotoxic effects that are mediated by Kupffer cell function.[35–38] Thus, thyroid status is an important factor in the development and progression of various types of liver diseases or in their prevention, including chemically-induced toxicity[3] and that involved after ischemia–reperfusion[7,8] or in cold organ storage in liver transplantation.[8]

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