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Liver Microsomal Parameters Related to Oxidative Stress and Antioxidant Systems in Hyperthyroid Rats Subjected to Acute Lindane Treatment

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Liver microsomal functions related to xenobiotic biotransformation and free radical production were studied in control rats and in animals subjected to L-3,3′,5-triiodothyronine (T_3) and/or lindane administration as possible mechanisms contributing to oxidative stress, in relation to the activity of enzymes (superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and glucose-6-phosphate dehydrogenase (G-6PDH)) and content of lipid-soluble vitamins (α-tocopherol, β-carotene, and lycopene) affording antioxidant protection. Lindane treatment in euthyroid rats at a dosage of 20 mg/kg did not modify the content of liver microsomal cytochromes P450 and b_5, the activity of NADPH-cytochrome P450 reductase and NADH-cytochrome b_5 reductase, and the production of superoxide radical ($O_2^{\bullet-}$), as well as antioxidant systems, except for the reduction in lycopene levels. Hyperthyroidism elicited a calorigenic response and increased specific and molecular activities of NADPH-cytochrome P450 reductase, $O_2^{\bullet-}$ generation, and G-6PDH activity, concomitantly with diminution in liver SOD and catalase activities and in α-tocopherol, β-carotene, and lycopene levels. The administration of lindane to hyperthyroid animals led to a further increase in the molecular activity of NADPH-cytochrome P450 reductase and in the $O_2^{\bullet-}$ production/SOD activity ratio, and decrease of hepatic α-tocopherol content, in a magnitude exceeding the sum of effects elicited by the separate treatments, as previously reported for reduced glutathione depletion. Collectively, these data support the contention that the increased susceptibility of the liver to the toxic effects of acute lindane treatment in hyperthyroid state is conditioned by potentiation of the hepatic oxidative stress status.

Keywords: Hyperthyroidism, lindane, oxidative stress, microsomal functions, antioxidant enzymes, antioxidant vitamins

INTRODUCTION

The disruption of the steady-state of cellular prooxidant molecules and antioxidants in favor of the prooxidants, potentially leading to damage,

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has been defined as oxidative stress.[1] The development of this redox disbalance in the liver can be accomplished by different conditions, including exposure to toxic xenobiotics such as lindane,[2] ischemia-reperfusion,[3] or hormonal dysfunctions such as hyperthyroidism.[4] These conditions, however, differ in that the primary mechanisms responsible for the production of reactive oxygen species (ROS) are different. In fact, lindane leads to high rates of superoxide radical ($O_2^{\bullet -}$) generation due to induction of the microsomal cytochrome P450-dependent biotransformation pathway,[2] whereas ischemia-reperfusion involves an enhanced production of ROS either by xanthine oxidase[5] or by activation of phagocytic cells within the liver.[6,7] In addition, thyroid hormone-induced prooxidant activity in the liver has been related to acceleration of cellular O_2 consumption, with increased production of microsomal, mitochondrial, and peroxisomal ROS[8,9] as well as cytosolic nitric oxide[10] in parenchymal cells, and enhanced respiratory burst activity in Kupffer cells.[11] Thus, the concurrence of such prooxidant conditions exacerbates the oxidative stress status of the liver and the resulting hepatocellular injury compared to the separate treatments, as demonstrated for hepatic ischemia-reperfusion[12] or acute lindane exposure[13] in hyperthyroid animals.

In the latter case, the administration of lindane to hyperthyroid animals resulted in marked increases in serum transaminases and extensive hepatocellular necrosis with presence of granulomas containing neutrophils, Kupffer cells, and lymphocytes.[13] In this situation, hyperthyroidism led to a significant decrease in the serum, liver, and adipose tissue levels of lindane, over values found in rats given lindane alone.[13] This effect of thyroid hormone may be due to enhancement in the hepatic microsomal biotransformation pathway,[14] although increases in cardiac output, hepatic and renal blood flow, and in the glomerular filtration rate[14] could affect the distribution and elimination of the insecticide. In this work, microsomal functions related to oxidative stress and xenobiotic biotransformation were determined in the liver under the influence of thyroid hormone and lindane to get insight into the hepatotoxic mechanisms involved, considering that this pathway produces a variety of reactive lindane-derived metabolites[15] in addition to ROS.[16] Results obtained were correlated with changes in the activity of hepatic enzymes affording antioxidant protection and in the content of lipid-soluble antioxidant vitamins.

MATERIALS AND METHODS

Male Sprague–Dawley rats fed *ad libitum* received daily intraperitoneal injections of 0.1 mg L-3,3′,5-triiodothyronine (T_3)/kg body weight or equivalent volumes of T_3 diluent (0.1 N NaOH) for 3 consecutive days. At the third day of hormone administration, animals were given either 20 mg lindane/kg or equivalent volumes of lindane vehicle (corn oil) intraperitoneally, forming 4 experimental groups: (a) control-control [CC], (b) control-lindane [CL], (c) T_3-control [T_3C], and (d) T_3-lindane [T_3L]. Determinations were performed 24 h after the last treatment in groups of animals exhibiting comparable values of body weight [CC, 259 ± 5 g ($n = 11$); CL, 258 ± 5 ($n = 12$); T_3C, 258 ± 4 ($n = 10$); T_3L, 256 ± 4 ($n = 10$)]. At this experimental time, the rectal temperature of the animals measured with a thermocouple was significantly increased by hyperthyroidism, independently of lindane treatment [CC (a), 36.7 ± 0.2°C ($n = 5$); CL (b), 37.1 ± 0.1 ($n = 7$); T_3C (c), 37.7 ± 0.1 ($n = 5$) ($P < 0.05$)^{a,b}; T_3L (d), 38.1 ± 0.2 ($n = 5$) ($P < 0.05$)^{a,b}], thus evidencing the calorigenic effect of T_3, known to involve a parallel enhancement in the rate of O_2 consumption in the liver.[8,11,13]

Determination of parameters in the liver tissue were performed after perfusion with ice-cold 0.9% w/v NaCl *in situ* to eliminate blood. The livers were removed, weighed, homogenized

(1 : 4) with 140 mM KCl, 10 mM potassium phosphate buffer pH 7.0, and microsomal and cytosolic fractions were obtained by conventional procedures.[17] Microsomes resuspended in 0.1 M sodium citrate buffer pH 7.6, containing 0.1 M KCl, 30% v/v glycerol, and 1 mM dithiotreitol,[17] were used to determine the content of cytochromes P450 and b_5[18] and the activity of NADPH-cytochrome P450 reductase[19] and NADH-cytochrome b_5 reductase.[20] Microsomes resuspended in 10 mM potassium phosphate buffer pH 7.0 containing 140 mM KCl were employed to measure superoxide radical ($O_2^{\bullet -}$) generation.[21] The activities of liver superoxide dismutase (SOD),[21] catalase,[22] glutathione peroxidase (GPx),[23] and glucose-6-phosphate dehydrogenase (G-6PDH)[24] were determined in the cytosolic fraction and expressed as units/mg of protein. The protein content of different subcellular fractions was assayed according to Lowry *et al.*[25] For the determination of α-tocopherol, β-carotene, and lycopene, lipid extracts were prepared by homogenizing approximately 100 mg of liver in 1 ml of water containing 0.05 ml of 10 mg/ml butylated hydroxytoluene to prevent autoxidation. After the addition of 1 ml of 0.1 M SDS, the mixture was vortexed for 30 s, mixed for 30 s with 2 ml of methanol, supplemented with 4 ml of hexane, vigorously vortexed for 1 min, and centrifuged for 5 min at $1000 \times g$ to separate the organic layer.[26] A 3 ml aliquot of the hexane layer was dried under N_2, and the residue was redissolved in 0.5 ml of methanol/ethanol (1/1 v/v) and filtered through a 0.22 μm-pore membrane. Quantitative measurements of α-tocopherol, β-carotene, and lycopene were made by HPLC[27] of lipid extracts using a Bioanalytical System electrochemical detector coupled to isocratic delivery system (Waters 510). Samples were injected through a Reodhyne system (loop: 20 μl) to a C8 Nova-Pak column, 4 μm, with dimensions of 3.9 × 150 mm. Separation was done using a flow rate of 1 ml/min of the mobile phase (20 mM lithium perchlorate in methanol/water 98/2 (v/v)). Electrochemical detection was done using an applied oxidation potential of 0.6 V. The observed retention times were 3.1, 5.1, and 8.9 min for α-tocopherol, lycopene, and β-carotene, respectively, and the amounts of each antioxidant were calculated by comparing the sample area with that of a known amount of standard. Standard solutions of α-tocopherol, β-carotene, and lycopene were prepared by dissolving the pure compounds in chloroform and diluted with ethanol to a final concentration of 1 μM, determined spectrophotometrically at 292 nm for α-tocopherol ($\varepsilon_{1\%} = 75.8$ $[M \times cm]^{-1}$), 453 nm for β-carotene ($\varepsilon_{1\%} = 2620$ $[M \times cm]^{-1}$), and 472 nm for lycopene ($\varepsilon_{1\%} = 3450$ $[M \times cm]^{-1}$).[28] Results are expressed as nmol/g liver.

All biochemical reagents used were obtained from Sigma Chemical Co. (St. Louis, MO). Values shown are means ± SEM for the indicated number of separate experiments. The statistical significance of difference among multiple groups was carried out by one-way ANOVA and the Tukey's test.

RESULTS

Administration of 20 mg of lindane/kg to fed rats did not alter liver microsomal cytochrome P450 content over control values, which was significantly depressed by T_3 treatment alone (37%) or by the joint T_3 and lindane administration (43%) (Figure 1A). NADPH-cytochrome P450 reductase specific activity was enhanced by 48% in T_3-treated rats and by 65% in animals subjected to the combined treatment over control values, parameter that was unaltered by lindane (Figure 1B). Furthermore, when this enzymatic activity is expressed in terms of the content of cytochrome P450, separate lindane and T_3 treatments enhanced the molecular activity of the reductase by 13 ($P > 0.05$) and 137% compared to controls, respectively, while the combined administration elicited a 191% increment

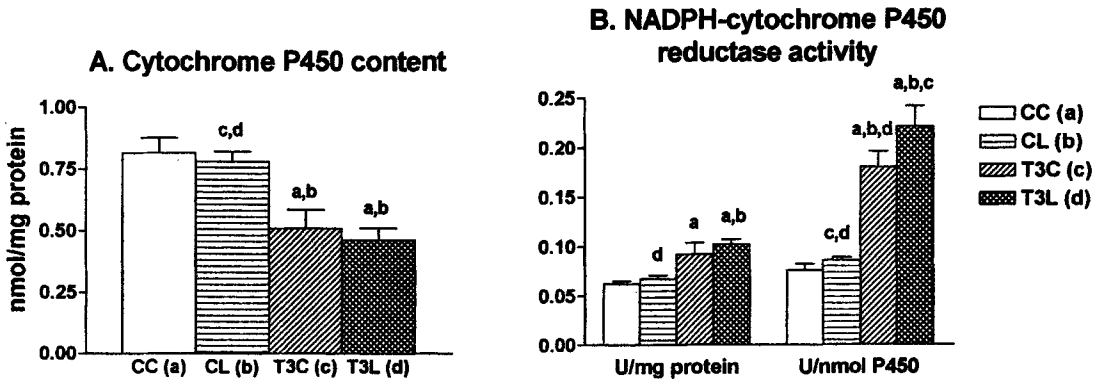

FIGURE 1 Cytochrome P450 content (A) and NADPH-cytochrome P450 reductase activity (B) in the liver of control rats and animals subjected to T_3 and/or lindane administration. Animals were divided into four groups: CC, control rats (a); CL, lindane-treated rats (b); T_3C, hyperthyroid rats (c); T_3L, hyperthyroid rats treated with lindane (d), as described in Materials and Methods. Values shown correspond to means ± SEM for 6–9 animals per experimental group. The significance of the differences between mean values ($P < 0.05$) was assessed by one-way ANOVA and the Tukey's test, and it is shown by the letters identifying each experimental group.

(Figure 1B). In these conditions, the microsomal content of cytochrome b_5 was also decreased by T_3 independently of lindane treatment [CC (a), 0.55 ± 0.05 ($n = 9$) nmol/mg protein; CL (b), 0.51 ± 0.03 ($n = 10$); T_3C (c), 0.39 ± 0.03 ($n = 9$)[a], T_3L (d), 0.39 ± 0.04 ($n = 8$)[a]], whereas the specific activity of NADH-cytochrome b_5 reductase was decreased by 30% only in the T_3L group [CC (a), 0.60 ± 0.03 ($n = 9$) U/mg protein; CL (b), 0.49 ± 0.03 ($n = 10$); T_3C (c), 0.51 ± 0.06 ($n = 9$); T_3L (d), 0.42 ± 0.05 ($n = 8$)[a]], being its molecular activity not altered by the separate and combined treatments over control values [CC (a), 1.09 ± 0.08 ($n = 9$) U/nmol cytochrome b_5; CL (b), 0.97 ± 0.04 ($n = 10$); T_3C (c), 1.29 ± 0.05 ($n = 9$)[b]; T_3L (d), 1.07 ± 0.11 ($n = 8$)]. Liver microsomal NADPH-dependent $O_2^{\bullet-}$ generation was not modified by lindane treatment compared to control values, being significantly increased by 56% and 79% in rats given T_3 alone or T_3 plus lindane, respectively (Figure 2A). Concomitantly, liver SOD activity remained unaltered after lindane administration and significantly depressed by T_3 (24%) or by T_3 plus lindane (25%) treatments (Figure 2B). From these data, it can be estimated that separate lindane (16%; $P > 0.05$) or T_3 (85%) treatments produced lower increments in the respective $O_2^{\bullet-}$ production/SOD activity ratios, over control values, than that elicited by the joint administration (138%) (Figure 2C).

The influence of separate lindane or T_3 treatments and their combined administration on the major antioxidant mechanisms of the liver is shown in Table I and Figure 3. As can be observed, lindane intoxication did not alter the activity of hepatic catalase, GPx, and G-6PDH compared to control values (Table I). Hyperthyroidism diminished liver catalase activity and increased that of G-6PDH independently of lindane treatment, whereas GPx activity in the different experimental groups was comparable to that in control rats (Table I). The hepatic content of α-tocopherol was not changed by lindane over control values, being significantly decreased by T_3 (37%) or by T_3 plus lindane (66%) treatments (Figure 3A). Hyperthyroidism also reduced the content of hepatic β-carotene (Figure 3B) and lycopene (Figure 3C) independently of lindane administration, whereas lindane treatment alone did not modify the content of β-carotene (Figure 3B) and significantly diminished (35%) that of lycopene (Figure 3C), compared to control values.

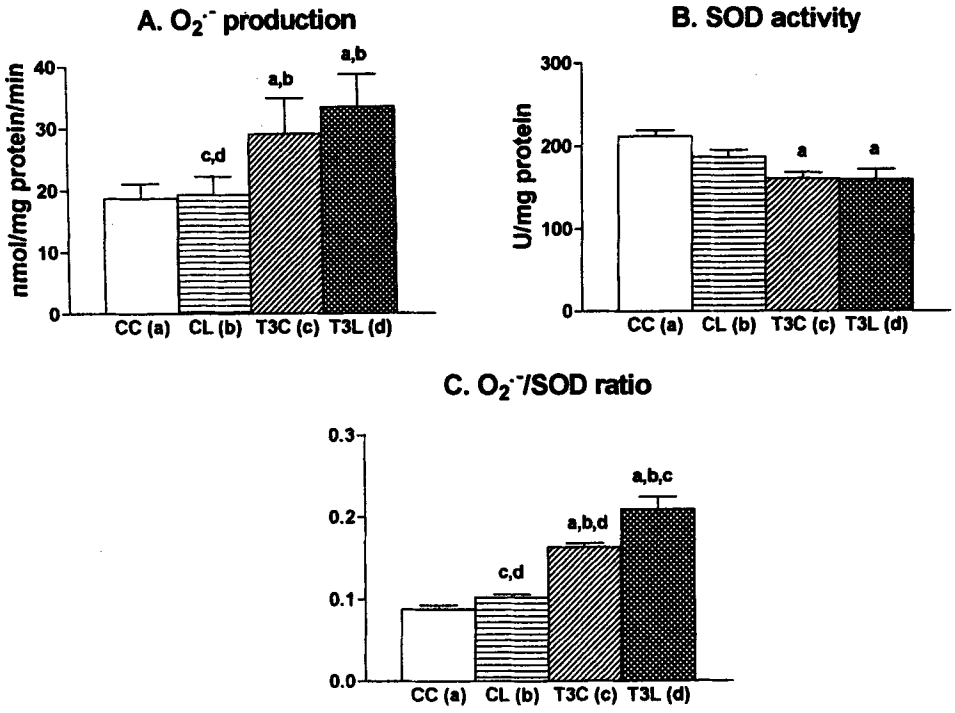

FIGURE 2 Microsomal superoxide radical ($O_2^{\bullet-}$) production (A), superoxide dismutase (SOD) activity (B), and $O_2^{\bullet-}$ production/SOD activity ratios (C) in the liver of control rats and animals subjected to T_3 and/or lindane administration. Animals were divided into four groups: CC, control rats (a); CL, lindane-treated rats (b); T_3C, hyperthyroid rats (c); T_3L, hyperthyroid rats treated with lindane (d), as described in Materials and Methods. Values shown correspond to means ± SEM for 4–6 animals per experimental group. The significance of the differences between mean values ($P < 0.05$) was assessed by one-way ANOVA and the Tukey's test, and it is shown by the letters identifying each experimental group.

TABLE I Activity of catalase, glutathione peroxidase (GPx), and glucose-6-phosphate dehydrogenase (G-6PDH) in the liver of control rats and animals subjected to T_3 and/or lindane administration

Enzymes	Experimental groups*			
	CC (a)	CL (b)	T_3C (c)	T_3L (d)
Catalase (U/mg protein)	389 ± 21 (8)†	394 ± 21 (7)^{c,d}	201 ± 22 (6)^{a,b}	155 ± 20 (5)^{a,b}
GPx (U/mg protein)	1.08 ± 0.18 (6)	0.99 ± 0.11 (4)	0.90 ± 0.05 (4)	0.85 ± 0.03 (4)
G-6PDH (mU/mg protein)	20.6 ± 3.0 (5)	25.8 ± 2.7 (5)^{c,d}	36.5 ± 5.4 (5)^{a,b}	37.0 ± 3.6 (6)^{a,b}

*CC, control rats (a); CL, lindane-treated rats (b); T_3C, hyperthyroid rats (c); T_3L, hyperthyroid rats treated with lindane (d), as described in Materials and Methods. †Results are means ± SEM for the number (n) of animals indicated in parentheses. The significance of the differences between mean values ($P < 0.05$) was assessed by one-way ANOVA and the Tukey's test, and it is shown by the letters identifying each experimental group.

DISCUSSION

Data presented indicate that lindane administration to fed rats at a dosage of 20 mg/kg did not modify liver microsomal parameters related to xenobiotic biotransformation and free radical activity, as well as antioxidant systems, except for the reduction in lycopene levels. However, a different situation is encountered when lindane is given to hyperthyroid animals, hormonal dysfunction known to increase the oxidative stress status of the liver.[4] In fact, the joint

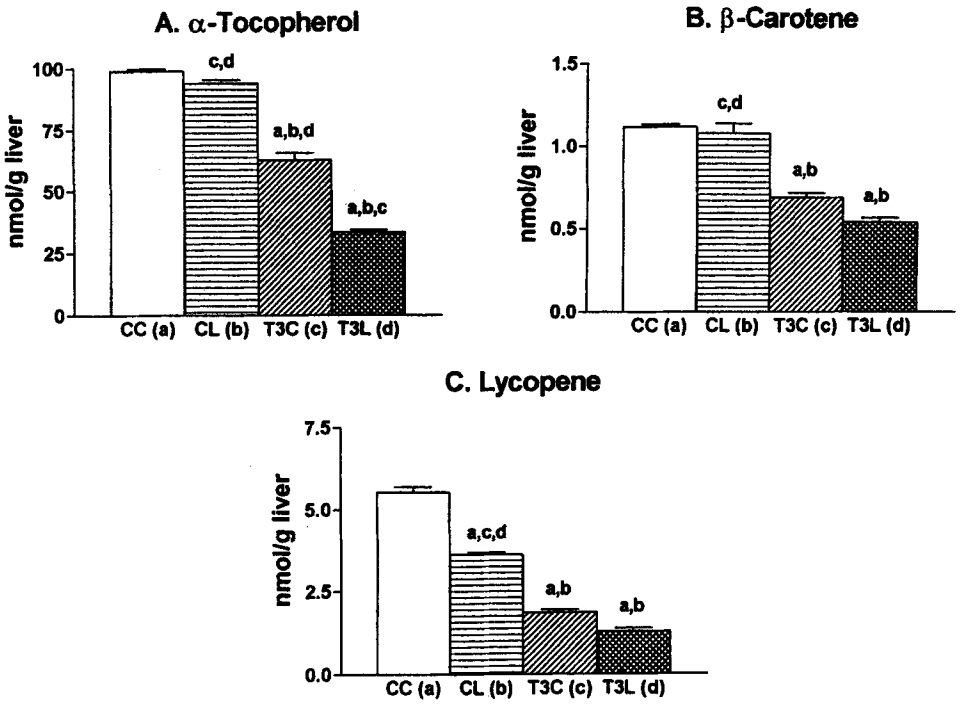

FIGURE 3 Content of α-tocopherol (A), β-carotene (B), and lycopene (C) in the liver of control rats and animals subjected to T_3 and/or lindane administration. Animals were divided into four groups: CC, control rats (a); CL, lindane-treated rats (b); T_3C, hyperthyroid rats (c); T_3L, hyperthyroid rats treated with lindane (d), as described in Materials and Methods. Values shown correspond to means ± SEM for 5–9 animals per experimental group. The significance of the differences between mean values ($P < 0.05$) was assessed by one-way ANOVA and the Tukey's test, and it is shown by the letters identifying each experimental group.

administration of T_3 and lindane led to a significant increase in the molecular activity of hepatic NADPH-cytochrome P450 reductase over that observed after the separate treatments, due to enhancement in the specific activity of the reductase and diminution in cytochrome P450 content. These changes, and the enhancement in the activity of the NADPH-generating enzyme G-6PDH by T_3 treatment, may imply greater rates of cytochrome P450 reduction and xenobiotic biotransformation, thus explaining the lower levels of lindane found in the serum, liver, and adipose tissue of hyperthyroid rats compared to those in euthyroid animals.[13] In agreement with this suggestion, thyroxin administration has been reported to increase the biotransformation of aminopyrine,[29] hexobarbital,[29] aniline,[30] and oxazolamine[30] in female euthyroid rats, as well as that of ethylmorphine, benzo(a)pyrene, and aniline in thyroidectomized rats,[31] and the microsomal reduction of Δ^4-3-ketosteroids.[32] In man, hypothyroidism caused an increase, and hyperthyroidism a decrease of antipyrine half-life as compared with that in the same patients after normalization of the thyroid status.[33] Discrepancies in the increasing effect of thyroid hormone on xenobiotic biotransformation seem to be related to the extent of the diminution of liver cytochrome P450 content, which in turn seems to be determined by the dose of hormone given and the period of treatment,[4] in addition to possible changes in cardiac, hepatic, and renal function.[14]

The formation of $O_2^{\bullet-}$ during the O_2 reductase activity of cytochrome P450 is attributed to autoxidation of the [Fe^{3+}-P450-O_2^-] complex either spontaneously or in the presence of certain xenobiotics.[16] Thus, enhancement in the rate of

cytochrome P450 reduction may also lead to increased rates of $O_2^{\bullet -}$, as found in liver microsomes from hyperthyroid rats independently of lindane treatment. This finding is paralleled by a depression in hepatic SOD activity, leading to a substantial increase in the $O_2^{\bullet -}$ production/SOD activity ratio in the liver of hyperthyroid rats subjected to lindane administration over values found after the separate treatments. The disbalance in the $O_2^{\bullet -}$ production/utilization equilibrium by the combined T_3-lindane treatment is likely to contribute to the enhancement in the oxidative stress status of the liver.[13] T_3-induced liver oxidative stress is conditioned by enhanced free radical activity in parenchymal cells[8–10] and in Kupffer cells,[10,11] coupled to a decrease in SOD (Figure 2B)[34] and catalase (Table I)[34] activities and depletion of glutathione (GSH).[34,35] In addition, data presented in Figure 3 show that hyperthyroidism also diminishes the content of the lipid-soluble antioxidants α-tocopherol, β-carotene, and lycopene over values found in euthyroid animals. Furthermore, liver α-tocopherol levels were further decreased by lindane administration to hyperthyroid rats compared to those found after the separate treatments (Figure 3A), as previously shown for GSH.[13] Collectively, data presented support the contention that the increased susceptibility of the liver to the toxic effects of lindane in hyperthyroid state is conditioned by potentiation of the oxidative stress status induced by thyroid hormone.[13] This seems to be accomplished by (a) enhancement in microsomal parameters related to lindane biotransformation, that may involve production of lindane-derived reactive metabolites, and in the $O_2^{\bullet -}$/SOD ratio, and (b) depletion of GSH[13] and α-tocopherol, in a magnitude exceeding the sum of the effects elicited by the separate treatments.

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