Lambda cyhalothrin toxicity induces alterations in lipogenic genes and inflammatory factors in rat liver

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Abstract
The present study aims to elucidate the molecular basis of lambda cyhalothrin (LCT) toxicity. Thirty-two mature male albino rats were randomly classified into four equal groups. The first group was orally administered normal saline, the second group was orally administered dimethylsulfoxide (DMSO). The third group was orally administered 1/100 LD\textsubscript{50} (6.12 mg/kg b. wt) of a commercial formulation containing 2.5\% LCT (i.e., a net dose LCT corresponding to 0.15 mg/kg b. wt). The fourth group was orally administered 1/100 LD\textsubscript{50} (0.64 mg/kg b. wt) of a pure form of LCT. The results indicated that exposure to LCT is capable of inducing an up-regulation in the mRNA expression levels of peroxisome proliferative activated receptor \(\alpha\) and \(\gamma\) (PPAR \(\alpha\) and PPAR \(\gamma\)), tumor necrosis factor (TNF-\(\alpha\)), fatty acid synthase (FAS) and sterol regulatory element binding protein-1c (SREBP-1c). Additionally, our study revealed a significant increase in serum levels of ALT, AST, ALP, \(\gamma\)GT as well as the inflammatory cytokines TNF-\(\alpha\) and monocyte chemoattractant protein-1 (MCP-1). A significant elevation in total lipids, total cholesterol, triacylglycerol, LDL-c and leptin with a corresponding significant decrease in HDL-c was also noted. Moreover, our results depicted that LCT treatment exhibits a significant increase in hepatic MDA levels concurrent with a significant decrease in GSH levels and the activities of CAT, SOD, and GPx. An immunohistochemical investigation also revealed a strong up-regulation of hepatic FAS in the LCT treated groups. The histopathological findings were marked by evidence in support of periportal fatty changes and interstitial aggregation of round cells.

Key Words: lambda cyhalothrin, lipogenic genes, liver, RT-PCR

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Introduction

Pyrethroids, major class of insecticides that alone account for more than one-third of the insecticide currently market in the world, are well known to be extremely lipophilic in nature\(^\text{[38]}\). These pesticides are extensively used in a wide variety of sectors including but not limited to crop and forestry protection, horticulture, hospitals, public health and the textile industry\(^\text{[12]}\).

Lambda cyhalothrin (LCT) is a synthetic type II pyrethroid with a broad spectrum insecticidal and acaricidal effects; it is mostly used in application where it is imperative to control insects and pest infestations\(^\text{[15]}\). Various studies have conclusively proved that LCT induces oxidative stress by increasing lipid peroxidation and altering the antioxidant defense system of the host body\(^\text{[16]}\). Additionally, it has been established that exposure to LCT is the causative agent responsible for decrease in body weight of male rabbits\(^\text{[38]}\).

Oxidative stress has the potential to cause an increase in the production of inflammatory transcription factors which are the key regulatory elements responsible for induction of the inflammatory response\(^\text{[24]}\). Peroxisome proliferative activated receptors (PPARs) are a class of nuclear receptors and ligand-dependent transcription factors that are well known as key regulators of lipid metabolism, immune regulation as well as cell differentiation\(^\text{[31]}\). Also, tumor necrosis factor alpha (TNF-\(\alpha\)) and other various other cytokines have been shown to be potent inhibitors of the adipogenesis process\(^\text{[40]}\).

The liver plays a critical role in lipid homeostasis by virtue of maintaining the balance between de novo lipid biosynthesis (lipogenesis and cholesterol biosynthesis) and mitochondrial \(\beta\) oxidation\(^\text{[34]}\). Analysis of a commercial variant of LCT has shown that 97.5% can be accounted for by the presence of additives such as biological activators or emulsifiers and wetting agents, including piperonyl butoxide which was known to increase the toxic effects of pyrethroid, and only 2.5% is composed of lambda cyhalothrin\(^\text{[35]}\). In light of the above mentioned data, the objective of the current study was to clarify the molecular aspects of the toxicological impact of LCT (commercial or pure form) on lipogenesis and immunoreaction as deduced from biochemical and histopathological studies.

2. Materials and methods

Chemical compounds: A commercial form of lambda cyhalothrin was obtained from the Pure Pharma Company for Medical and Chemicals, Egypt (lambda 2.5% EC). The composition (per 1) was as follows: 2.5% lambda cyhalothrin, 6% piperonyl butoxide, 2.5% Geronol Ms and slovesso up to 100%. The liquid was clear straw colored in appearance. LCT is also known by the following synonyms and trade names: Charge, Excaliber, Grenade, Hallmark, Icon, Karate, Saber, Sentinel and Samuri. The pure form of lambda cyhalothrin was imported by the Egyptian International Center for import from Chemie GmbH, Riedstrasse 2.D89555 Steinheim, Germany. Pure LCT has a white powdery appearance. The chemical formula is as follows: alpha-cyano-3phenoxybenzy3-(2-chloro-3,3,3 trifluoropropenyl)-2,2,dimethylcyclo- propane carboxylate.

Animal and treatment: All rats were obtained from the Animal House at the Faculty of Veterinary Medicine, Zagazig University, Sharkia, Egypt. The rats were given \textit{ad libitum} access to food and water with a 12 h/12 h dark-light cycle. All animals included in the study were allowed a 15 day time period for adapting to standard cages under controlled conditions. All animal related procedures were conducted in accordance with guidelines prescribed by the ethical committee of Zagazig University. The current study was conducted on 32 male Wistar albino rats of age four months, and weighing 180–200 g. The test subjects were randomly segregated into four equal groups. The first group was orally
administered normal saline and served as a negative control. The second group was orally administered dimethylsulphoxide (DMSO) which known to have no hepatotoxic effect\(^\text{22}\); the DMSO used was procured from Sigma-Aldrich Co. (St. Louis, MO, USA) and used as lambda cyhalothrin solvent. The third group of animals was orally administered 1/100 LD\(_{50}\) of commercially available lambda cyhalothrin containing 2.5% LCT (6.12 mg/kg b. wt) as determined previously by Celik et al\(^\text{10}\) (i.e., net dose LCT: 0.15 mg/kg b. wt). The fourth group was administered 1/100 LD\(_{50}\) of the pure form of lambda cyhalothrin (0.64 mg/kg b. wt) as per the USEPA\(^\text{33}\). The above mentioned solutions were administered to the selected test group by oral application through a gastric lavage procedure conducted on alternate days for a period of two months.

**Sampling:** At the end of the experiment, blood samples were drawn from the medial canthus of the eyes into chilled non-heparinized tubes and centrifuged at 5000 rpm for 20 min at 4°C. The separated sera were frozen at −20°C for biochemical analysis. Rats were sacrificed by decapitation and liver samples of both the control and treated groups were taken and immediately preserved by snap freezing in liquid nitrogen. The livers samples of both control and treated groups were taken and immediately preserved in liquid nitrogen container and subsequently stored at −80°C for semi-quantitative RT-PCR analysis. To monitor the hepatic antioxidant status, liver samples were weighed and homogenized in chilled potassium chloride (1.17%). A homogenizer from Potter–Elvehjem was used for this purpose. Liver specimens from all groups were fixed in 10% neutral buffered formalin solution for immunohistochemical localization of FAS and histopathological examination.

**Semi-quantitative RT-PCR:** A 100 mg liver tissue specimen was collected for total RNA extraction. The sample was frozen in liquid nitrogen and subsequently stored in 1 mL Qiazol (QIAGEN Inc., Valencia, CA) at −80°C. Frozen samples were homogenized using a Polytron 300 D Homogenizer (Brinkman Instruments, Westbury, NY) and 0.3 mL chloroform was added to the homogenate. The mixture was briefly shaken for 30 s before centrifugation at 12,500 rpm for 20 min at 4°C. The supernatant layer was transferred to fresh tubes and an equal volume of isopropanol was added to the samples, mixed for 15 s and centrifuged at 12,500 rpm for 15 min at 4°C. RNA pellets were washed with 70% ethanol, dried briefly before being dissolved in diethylpyrocarbonate (DEPC) treated water.

The quality of RNA was estimated by spectroscopic measurement at 260/280 nm and confirmed by denaturing gel electrophoresis. For the synthesis of cDNA, a mixture containing 2 μg total RNA and 0.5 ng oligo dT primer in 11 μL sterilized DEPC water was incubated in a thermal cycler (model 2720; Applied Biosystems, Foster City, CA) at 65°C for 10 min to ensure complete denaturation. Subsequently, 4 μL of 5X RT-buffer, 2 μL of 10 mM dNTPs and 100 U Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase (SibEnzyme Ltd. Ak, Novosibirsk, Russia) was added and the total volume was made up to 20 μL by the addition of DEPC-treated water. The mixture was then re-incubated in the thermal cycler at 37°C for 1 h. A subsequent incubation at 90°C for 10 min was conducted to ensure inactivation of the enzyme. Equivalent quantities of the reverse transcribed products (1 μg cDNA) were subjected to PCR amplification. A total volume of 25 μL containing PCR master mix (Promega Corporation, Madison, WI) and gene-specific primers (Table 1) that had been designed using the Oligo 4 computer program and synthesized by Macrogen (Macrogen Company, Gasa-dong, Geum-Cheong. Korea) were used to this purpose. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA was used as an internal control for PCR. As the initial step of the PCR amplification procedure, the sample was denatured by at 95°C for 5 min. This was followed by thermal cycling consisting
of 30 s at 95°C (denaturing temperature), annealing temperature (dependent on genes under study; Table 1) and 60 sec at 72°C (extension temperature). A final extension step was included by incubating sample at 72°C for 7 min. A single major band corresponding to the desired amplicon was detected by electrophoresis on a 1.5% agarose/ethidium bromide (0.25 μg/mL) gel. The PCR products were visualized under UV light and photographed using gel documentation system. The intensities of the bands were quantified densitometrically using the public domain NIH Image program (National Institutes of Health, Bethesda, Maryland).

**Biochemical analysis:** The sera were analyzed for estimation of activity of aspartate aminotransferase (AST; EC 2.6.1.1), alanine aminotransferase (ALT; EC 2.6.1.2), gamma glutamyl transferase (γ GT; EC 2.3.2.2), alkaline phosphatase (ALP; EC 3.1.3.1) and total lipids as described by Frings et al. Total cholesterol, quantification of triacylglycerol, high density lipoprotein-c (HDL-c) and low density lipoprotein-c (LDL-c) were determined as described by Friedwald et al. Leptin concentration was measured by using commercial ELISA kits for rats. Quantitative determination of TNF-α in rat serum was done using ELISA kits (catalog no.45-TNFR-E01.1, ALPCO immunoassays, Georgia, USA). Manufactures instructions were followed to this purpose.

The expression of monocyte chemoattractant protein (MCP-1) in the serum was quantitatively evaluated as per the instructions provided with the commercially available ELISA-based kit (catalog no. MBS 266051, Rapid bio lab, USA) and as detailed previously by Brady et al. Subsequent to homogenization of the hepatic tissues, the nuclear debris was separated by centrifugation at 8000 × g, 4°C for 5 min. The supernatant was used for the purpose of assaying malondialdehyde (MDA), lipid peroxidation marker, CAT (EC 1.11.1.6), SOD (EC 1.15.1.1), GPx (EC 1.11.1.9) and reduced glutathione (GSH) content using a Shimadzu spectrophotometer (UV 120-02).

**Histopathological examination:** Liver specimens were processed as per routine by dehydration in gradual ethanol (70–100%), followed by clearing in xylene and embedding in paraffin. The paraffin sections of 5 microns thickness were prepared and stained with hematoxylin and eosin (H&E) dyes as per protocol described by Wilson and Gamble.

**Immunohistochemical examination of Fatty acid synthase (FAS):** Immunohistochemical analysis was used to examine the distribution of the FAS in liver samples. Subsequent to deparaffinization, the sections (thickness: 5 μm) were treated with 3% H2O2 for 10 min to order to achieve inactivation of the peroxidases. Antigen retrieval was done by heating the tissue samples in 10 mM citrate buffer at 121°C for 30 min. The samples were then blocked in 5% normal serum for 20 min followed by incubation with a rabbit polyclonal anti-FAS primary antibody (1 : 100; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) in phosphate-buffered saline for 14-16 h at 4°C. After three extensive washes with PBS, the liver sections were incubated with IgG biotin-conjugated anti-rabbit secondary antibody (1 : 2000; Santa Cruz Biotechnology, Inc.) for 20 min at 32°C. Following incubation with horseradish peroxidase-labeled streptavidin, antibody binding was visualized using 3-3 diaminobenzidine tetrahydrochloride and the sections were counterstained with hematoxylin. For the purpose of defining a suitable negative control, a specimen was processed using the protocol outlined above except for the addition of the primary antibody. For microscopic analysis, the presence of brownish-yellow cytoplasmic granules was recognized as positive staining. Images were captured using a Canon power Shot digital camera (Canon, Inc., Tokyo, Japan).

**Statistical analysis:** Data obtained from the study is expressed as mean values ± SE. SPSS statistical version 21 software package (SPSS,
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Inc, USA) was used to analyze the results by one-way analysis of variance (ANOVA). Duncan’s test was used for making multiple comparisons amongst the groups for testing the inter-grouping homogeneity.

**Results**

**Effect on mRNA levels of inflammatory and lipogenic genes in hepatic tissues**

The current study revealed that there is a significant up-regulation of PPAR-α, PPAR-γ and TNF-α mRNA levels in both the treated groups when compared with data obtained from the control set. This difference is more obvious in the group that was treated with a pure form of LCT especially with respect to PPAR-α and PPAR-γ expression. In contrast, the increase in the TNF-α mRNA levels was more pronounced and significant in case of the group that was treated with the commercially available lambda cyhalothrin as compared to the control group (Fig. 1A, B, and C). The mRNA levels of FAS and SREBP-1c were observed to be significantly elevated when the commercial LCT treated group was compared with the control group (Fig. 2 A and B).

**Effect on serum biochemical parameters**

**Liver marker enzymes**

The data depicted in Table 2 demonstrate that, compared to control group, the groups treated with the commercial and pure forms of LCT (6.12 mg/kg and 0.64 mg/kg, respectively) showed a significant increase ($P < 0.05$) in serum levels of ALT, AST, γGT and ALP enzymes. Also statistical analysis clearly demonstrated that the observed increase was significant when ALT levels of both treated groups were compared with each other.

**Inflammatory cytokines**

Animals that were administered commercially and pure doses of LCT displayed a significant increase ($P < 0.05$) in serum levels of TNF-α and MCP-1 when compared with the control groups. However, this increase was not statistically significant when the two treated groups were
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compared with each other (Table 2).

**Lipid profile**

The results enumerated in Table 3 show that in comparison to the control group there was an obvious and significant increase ($P < 0.05$) in the serum level of total lipids, total cholesterol, triacylglycerol, LDL-c and leptin in both the treated groups, but, when the two treated groups were compared with each other it was observed.

Fig. 1. The effects of commercial and pure forms of lambda cyhalothrin administered every other day for two months at dosage levels of 6.12 mg/kg body weight and 0.64 mg/kg body weight, respectively, on PPAR-α (A), PPAR-γ (B) and TNF-α (C) mRNA expression in hepatic tissues of rats. The densitometric analysis of three different rats of each group.
that the increase in the level of total lipids and leptin was not statistically significant. However, it was seen that the level of HDL-c showed a significant decrease ($P < 0.05$) in both the treated groups as compared to the control rats.

**Oxidative and antioxidant status in liver homogenate**

The data recorded in Table 4 revealed that in comparison to the control group a significant rise ($P < 0.05$) in MDA concentration was observed in the groups treated with the commercial or pure form of LCT. Also, it was observed that the hepatic GSH level and the activities of CAT, SOD, and GPx were significantly decreased in both treated groups when contrasted with the control rats.

**Histopathological findings**

Microscopic examination of the liver revealed the presence of normal looking hepatocytes and a normal sinusoidal architecture in control group (Fig. 3A). In sharp contrast, the liver of rats treated with commercial LCT exhibited severe congestion of hepatic blood vessels and sinusoids with pressure atrophy of the hepatic cords (Fig. 3B). Multiple areas of coagulative necrosis focally replaced with round cells were also detected (Fig. 3C). Also, extravasated erythrocytes were observed in the adjacent areas. Interstitial aggregations of round cells were seen scattered throughout the hepatic tissue (Fig. 3D). Some portal areas were observed to be widened with a congested portal vein; bile ducts were proliferated and showed presence of round cell infiltrations as well as some fibroblast proliferations (Fig. 3E). Severe hydropic degeneration and diffuse periportal fatty change (macrovesicular type) was also visualized (Fig. 3F). Interestingly, the hepatic lesions in rats that received pure lambda cyhalothrin were milder by comparison. Examination of the liver revealed a portal area with severe congestion of portal blood vessels and round cells (Fig. 3G). The fatty change was rarely seen in individual cells besides round cells in the portal areas and the numerous binucleated hepatocytes (Fig. 3H). Few interstitial aggregations of round cells (Fig. 3I) were also detected in

![Fig. 2. The Effect of commercial and pure forms of lambda cyhalothrin administered every other day for two months at a dosage of 6.12 mg/kg body weight and 0.64 mg/kg body weight respectively, on FAS (A) and SREBP-1c (B) mRNA expression in hepatic tissues of rats. The densitometric analysis of three different rats of each group.](image-url)
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Addition to a moderate level of hydropic degeneration.

Immunohistochemical detection of liver FAS

Our results revealed a weak cytoplasmic reaction in the hepatocytes of the control and
Fig. 3. A-Liver of control rats showing normal hepatocytes and sinusoidal architectures. HE x400. B-Liver of a commercial lambda cyhalothrin treated animals showing severe congestion of hepatic blood vessels and sinusoids with pressure atrophy of the hepatic cords. HE x400. C-Liver of a commercial lambda cyhalothrin treated rats showing areas of coagulative necrosis focally replaced with round cells. HE x400. D-Liver of a commercial lambda cyhalothrin treated rats showing interstitial aggregation of round cells. HE x400. E-Liver of a commercial lambda cyhalothrin treated animals showing portal area with round cells infiltrations, congested blood vessel (c) and hyperplasia in the bile duct epithelium. HE x400. F-Liver of commercial lambda cyhalothrin treated group showing diffuse periportal fatty change. HE x400. G-Liver of pure lambda cyhalothrin treated rats showing congestion of portal B.Vs and round cells. HE x400. H-Liver of pure lambda cyhalothrin treated rats showing rare fatty change, round cells in the portal area and numerous binucleated hepatocytes. HE x400. I-Liver of pure lambda cyhalothrin treated rats showing few interstitial aggregations of round cells. HE x400.
DMSO-treated groups (Fig. 4A, B) whereas a strong FAS reaction was observed when the commercially treated group was analyzed (Fig. 4C) and a moderate reaction was observed when the same was analyzed for the group treated with the pure form of LCT (Fig. 4D).

Discussion

LCT is a synthetic pyrethroid with a broad spectrum of insecticidal and acaricidal activities. In spite of its extremely popular use, very little is known about the adverse effects of LCT especially those related to its impact on the molecular mechanism of immunotoxicity and lipogenesis. Peroxisome proliferation activated receptors (PPARs) are ligand-dependent transcription factors that are well known as key regulators and modulators of lipid homeostasis and inflammation. This study has clearly demonstrated that in case of mature male albino rats, administration of LCT, either in its commercial or pure form (6.12 & 0.64 mg/kg b. wt respectively), results in an up-regulation of the hepatic inflammatory cytokine genes. It was observed that in comparison to the control group, PPAR-α and PPAR-γ mRNA expression was significantly up-regulated in the group treated with pure LCT. On the other hand, TNF-α mRNA expression was significantly elevated in the rats treated with commercial LCT (Fig. 1 A, B, C). Our findings, illustrating a significant rise in serum levels of TNF-α and MCP-1 in both the treated groups as compared to controls, are in accord with previously reported data (Table 2).
Our results corroborate with the findings of Lee et al.\(^{24}\) who reported that the insecticide chlorpyrifos induces an increase in inflammatory genes such as PPAR-\(\gamma\) and TNF-\(\alpha\) in Sy5-y-cells. In addition, it has also been reported that deltamethrin pyrethroid significantly increases the serum levels of TNF-\(\alpha\) in male albino rats\(^{13}\).

Contrary to the results presented above, Costa et al.\(^{11}\) reported a reduction in inflammatory cytokine levels when they studied the immunotoxic effects of the synthetic pyrethroid \(\alpha\) cypermethrin. It is our hypothesis that this discrepancy between results can be accounted for by the type of pyrethroid as well as dose and time of administration. Our findings regarding an increase in inflammatory cytokine production upon exposure to LCT is supported by our histopathological findings, which show portal areas with round cells infiltration and interstitial aggregation of round cells.

Regarding the effects of LCT on the expression of hepatic lipogenic genes, our study noted that, in comparison to the controls, there was a significant up-regulation in the expression levels of mRNAs related to the FAS and SREBP-1c systems in the liver of both the treated groups. Also, the up-regulation was more evident in the group that was treated with the commercial LCT; this finding is also supported by our immunohistochemical findings regarding FAS (Fig. 4A-D). In corroborate of the above mentioned finding, it is well known in the literature that activation of PPARs results in an increased expression of a variety of gene families especially those that regulate lipid metabolism\(^{26}\).

The strength effect of commercial form of LCT on lipogenic genes and inflammatory cytokines may relay on the moderate rate of hydrolysis of its alcohol moiety (cyano-3- phenoxybenzyl group)\(^{38}\). Our results regarding the up-regulation of lipogenic genes are in agreement with those presented by Jin et al.\(^{23}\) who reported that when mice were exposed to cypermethrin pyrethroid, it elevated liver mRNA levels of a number of key genes involved in fat metabolism and fatty acid synthesis. Male rabbits treated with LCT exhibit a significant decrease in body weight resulting from a lowering of food intake either due to a direct effect of pesticide on somatic cells or because of its effect on the central nervous system\(^{39}\). The observed accumulation of hepatic total lipid in the rats stimulated weightlessness probably as a result of decreased lipolysis in combination with excessive glycogen breakdown that led to glucose being used as a substrate for lipogenesis\(^{21}\). The above mentioned results reinforce our findings regarding the increase in lipogenic factors observed as a result of LCT toxicity; it also substantiates our immunohistochemical findings regarding hepatic FAS.

Contrary to the evidence presented till now, a study conducted by Armstrong et al\(^{4}\) reported that deltamethrin pyrethroid caused a decrease in the mRNA expression levels of lipogenic genes including that of the FAS and SREBP-1c systems in male mice. An in depth analysis of all datasets lead us to speculate that this difference can be attributed to the examined pyrethroid, dose, duration and the experimental animal used. Regarding the status of the lipogenesis machinery, our findings indicated towards a significant elevation in the serum levels of lipids including total lipids, total cholesterol, triacylglycerol and LDL-c with a significant decrease in HDL-c levels being observed in case of the LCT treated groups (Table 2). In agreement with above, a previous study reported by Bhushan et al\(^{6}\) also claimed that cypermethrin treatment in rats led to an increase in total lipids and total cholesterol in the hepatic homogenate.

Interestingly, in accord with the substantial increase in the serum level of MCP-1 previously mentioned in our study, a significant rise in the serum levels of leptin in both the groups of animals treated with LCT is also evidenced. This is supported by similar findings reported by Yamagishi et al\(^{37}\) who postulated that an increase in leptin levels results in stimulation of MCP-1 overexpression via increased ROS generation. This is confirmed by the results of our antioxidant
analysis (Table 4) and also by the study results reported by Ben Abdallah et al\textsuperscript{4} who observed that male rats treated with LCT display a decreased level of GSH and reduced activities of CAT, GPx and GST.

Our histopathological findings which presented evidence of hepatic fat infiltration and periportal fatty changes (Fig. 3) also confirm the aforementioned study. The findings also correlate with similar findings reported by Basir et al\textsuperscript{3} who investigated the pathological effects of LCT in case of female rabbits. In this respect, the liver impairment was evaluated by analyzing the alteration in the levels of liver enzymes which revealed a significant rise in serum concentrations of ALT, AST, ALP and γ GT in the LCT treated groups. Also, the liver enzymes of the cypermethrin treated rabbits showed a significant increase\textsuperscript{12}.

Piperonyl butoxide, a chemical that is known to act synergistically with the pyrethrins by inhibiting the mixed function oxidase activity, is widely used as a pyrethroid additive\textsuperscript{9}. Due to inhibition of enzymatic detoxification, the parent compound is seen to persist for a longer time period\textsuperscript{35}. This evidence confirms the toxic effects of piperonyl butoxide in LCT commercial formulations.

Conclusion

In conclusion, the data presented by this study suggests that exposure to LCT, either in its commercial form or in its pure state, is capable of significantly inducting and elevating levels of hepatic mRNAs especially those related to the inflammatory and lipogenic genes. A significant elevation in the serum levels of liver enzymes, inflammatory cytokines, lipid profile and hepatic MDA with decreased antioxidant enzyme activities was also observed. This result is corroborated by the well marked hepatic histopathological perturbations observed under microscopic analysis and also by the immunohistochemical examination of fatty acid synthase in which, it was noted, that changes were more obvious in the group that was treated with the commercial form of LCT as compared to the pure LCT-treated group as the commercial version contains additives that lead to an increase in toxicity.

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References


