Histopathological Changes in Liver of Albino Rat Exposed by Endosulfan

Noor Al-Huda R. Mayea[1]* and Mukhtar K. Haba[2]

[1]Department Medical laboratory techniques, College of Health and Medical Technology, Middle Technical University, Baghdad, Iraq

[2]Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq

*Corresponding Author. E-mail: nooralhuda_radhi@mtu.edu.iq (N.M.); Tel. +964 772 178 9517.

ABSTRACT

Despite its extremely harmful effects, endosulfan, an organochlorine insecticide, is a commonly used pesticide in agriculture. Consequently, the study's goal was to ascertain how endosulfan affected the albino rats' liver' histology. Endosulfan was administered orally to male rats at low, medium, and high doses (3.5, 7, and 10.5 mg/kg) for a duration of 15, 30, and 45 days. Increased of ALP and AST, mild diltation and congestion progressed to severe necrosis, cellular swelling, infiltration of kupffer cells, degeneration and disarrangement of hepatic cords at higher doses and prolonged exposure. In conclusion, endosulfan damaged and was harmful to liver. This pesticide caused oxidative stress in liver; Oxidative stress was shown to be increase in oxygen free radical production. Thus, that lead to be alteration in histology and function of liver male albino rat.

Key words: Endosulfan, Histological changes, Liver, Toxicity.

INTRODUCTION

Pesticides are a broad category of chemicals that are used extensively in public health, and agriculture to prevent and control pests. It is believed that these chemicals may have negative health consequences (1,2,3). Many pesticides are suspected of acting as endocrine disruptor chemicals (EDCs) and potentially dangerous to those exposed, even at extremely low exposure levels (4,5). Exposure to EDCs during the prenatal and postnatal phases of development until puberty can have a negative impact later in life by interfering with the physiology of normal endocrine-regulated events (6,7).

Endosulfan, an organochlorine (OC) insecticide, is a widely used agricultural pesticide, despite its life-threatening toxic effects (8,9). This insecticide is applied to control a wide variety of pests that infect of the crops including hazelnut, tea, a wide group of fruits, as well as cereals, maize and else grains (10). Despite that, the endosulfan utility has been reduced because of its lengthy stability in the agricultural fields, as well as, it strongly toxic to fish in the aquatic ecosystems, and harmful effects to farmers (11,12,13). According to studies, Endosulfan exposure may have negative health effects such as endocrine disruption

(6), neurotoxicity, genotoxicity, infertility, spontaneous miscarriages, and hepatotoxicity (7). In several species, endosulfan may have these effects by different ways, the decreases in antioxidant defenses leading to oxidative stress caused by the build-up of reactive oxygen species (ROS) could be one mechanism of toxicity. Overproduction of free radicals damages fatty acids and proteins found in cell membranes, impairing their functionality irreversibly.

Many pesticides have cytotoxic effects on non-target organisms, such as causing reactive oxygen species (ROS) to be produced, which can damage proteins, lipids, and DNA in cells (14). Rats exposed to EDS resulted in oxidative liver damage brought on by ROS production in the tissue (15).

MATERIALS AND METHODS

Sampling

Thirty -two sexually mature laboratory males Albino rats, aged (6-8 week) with an average weight of about (200-230) gm were used in current study. The thirty –two rats were divided into four groups, each group contains 8 rats. Animals were bought from the Biotechnology Research Center/Al-Nahrain University, and housed in the animal house of the Collage of Science for Woman, University of Baghdad, they were kept in plastic cages (40x25x15 cm) with controlled room temperature 25 C, under laboratory conditions, and12:12 dark and light cycle. Rats were provided with water and food *ad-libitum*.

Groups of Experimental Animals:

The animals were divided into four groups according to the doses of Endosulfan given and duration (15,30,45 days) were treated as follows

First Group(G1):

The group included (8) animals as a control, and each of them was dosed with (1) ml of normal saline solution only for 45 days- via daily oral administration.

Second group(G2):

The group included (8) animals each of them was dosed with (1) ml at a concentration of 3.5mg/kg for 45days- via daily oral administration

Third group(G3):

The group included (8) animals that were dosed with the insecticide at a dose of (1) ml at a concentration of 7mg/kg for 45days-via daily oral administration.

Fourth group(G4):

The group included (8) animals that were dosed with the insecticide at a dose of (1) ml at a concentration of 10.5mg/kg for 45days- via daily oral administration.

At the end of each period (15,30,45 days), male rats have been sacrificed, and the Liver obtained from each groups were fixed with10% buffered formalin, liver tissues embedded with paraffin. After routine processing, paraffin sections were cut into 5 µm thickness and stained with haematoxylin and eosin(16).

Liver function tests

Liver enzymes (Aminotransferase) level measurement

The procedure was performed in order to determine ALP, AST levels in all groups (G1, G2, G3, and G4). AST and ALP mindray kit was used to perform these testes.

Statistical Analysis:

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means in this study. ($P < 0.05$) was considered statistically significant(17).

RESULT

Effect Endosulfan on ALP (alkaline phosphatase)

Table 1 presents the effect of different doses (control, 3.5 mg/kg, 7 mg/kg, and 10.5 mg/kg) on ALP (alkaline phosphatase) levels over three time periods (15 days, 30 days, and 45 days). The ALP levels in the control group remained the lowest across all time periods, while the treated groups showed increasing ALP levels as the dosage increased. The highest ALP levels were found in the 10.5 mg/kg group, which also showed a significant decrease in ALP levels between 15 and 45 days. The overall results suggest that the 10.5 mg/kg dosage significantly affects ALP levels over time, while lower doses did not show significant changes across time, indicating a dose-dependent effect on ALP levels.

Table 1: Effect of doses and period in ALP(U/L)

Groups	15 days	30days	45days	LSD value
Control	72.23 ±3.47 C a	77.42 ±2.98 C a	80.40 ±3.75 C a	11.47 NS
3.5mg/kg	151.02 ±8.06 B a	154.65 ±8.45 B a	158.55 ±7.91 B a	14.92 NS
7mg/kg	166.25 ±8.35 B a	176.50 ±9.02 B a	189.35 ±9.43 B a	24.76 NS
10.5mg/kg	198.70 ±10.02 A b	221.76 ±11.27 A ab	236.15 ±12.7 A a	31.35 *
LSD value	27.81 *	35.06 *	39.16 *	---
Means with different big letters in the same column and small letters in the same				

row are significantly different. * (P≤0.05).

Effect Endosulfan on AST (aspartate aminotransferase)

Table 2 illustrates the effect of different doses (control, 3.5 mg/kg, 7 mg/kg, and 10.5 mg/kg) on AST (aspartate aminotransferase) levels across three time periods (15 days, 30 days, and 45 days). However, in the 10.5 mg/kg group, a significant difference ($P \leq 0.05$) was observed, showing a notable decrease in AST levels from 45 to 15 days. Additionally, AST levels are dose-dependent, with the control group having the lowest levels and the 10.5 mg/kg group showing the highest values. The overall results suggest that higher doses, particularly 10.5 mg/kg, have a significant impact on increasing AST levels, while the control and lower doses showed minimal changes across the time periods.

Table 2: Effect of Endosulfan doses and period in AST(U/L)

Groups	15 days	30days	45days	LSD value
Control	23.53 ±1.26 C a	24.67 ±1.54 C a	26.07 ±1.47 C a	9.73 NS
3.5mg/kg	48.95 ±2.85 B a	55.08 ±2.78 BC a	59.95 ±2.95 B a	12.48 NS
7mg/kg	65.25 ±2.96 AB a	68.27 ±3.06 AB a	75.98 ±3.17 B a	12.03 NS
10.5mg/kg	85.36 ±3.57 A b	94.68 ±4.22 A b	119.63 ±6.51 A a	18.52 *
LSD value	21.08 *	27.55 *	30.04 *	---
Means with different big letters in the same column and small letters in the same row are significantly different. * (P≤0.05).				

Histopathological Changes

Control group

The liver is surrounded by a capsule composed of collagenous connective tissue. The capsule sends trabeculae into liver parenchyma divided the organ into lobules. The lobule is hexagonal prism in shape with a branch of the hepatic vein located in the center of the lobule (central vein). Hepatic cells (Hepatocytes) arranged as a plates radiate from the center vein toward the periphery of the lobule named hepatic cords separated by sinusoids. normal histological appearance indicates well-preserved lobular architecture (Figure 1).

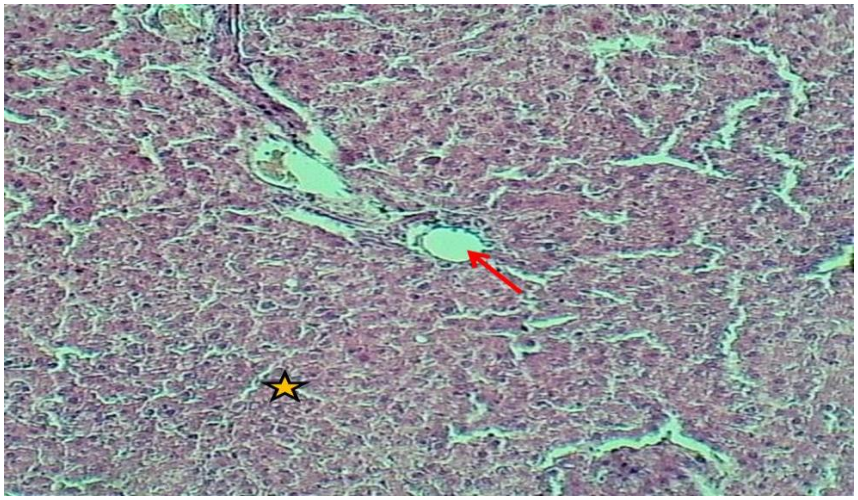

Figure 1: Section of liver (control) show normal portal vein (red and arrow) & hepatic cords (asterisk) H&E stain.100x.

Exposed group for 15 days.

The effect of Endosulfan pesticide on the liver of exposed rats showed more obvious histopathological changes compared to the control group. The exposed group with concentration of 3.5mg/kg showed noticeable changes represented by mild dilation with congestion of central vein, cellular swelling and necrosis of some hepatocytes, Increase of Kupher cells inside the sinusoids. In the exposed group with concentration of 7mg/kg, the damage has increased significantly, as it is represented by sever generalize cellular swelling with necrosis within hepatocytes. The group exposed with concentration 10.5mg/kg the damage is shown sever dilation of central vein, sever generalize cellular swelling of hepatocytes with sever necrosis (Figures 2,3).

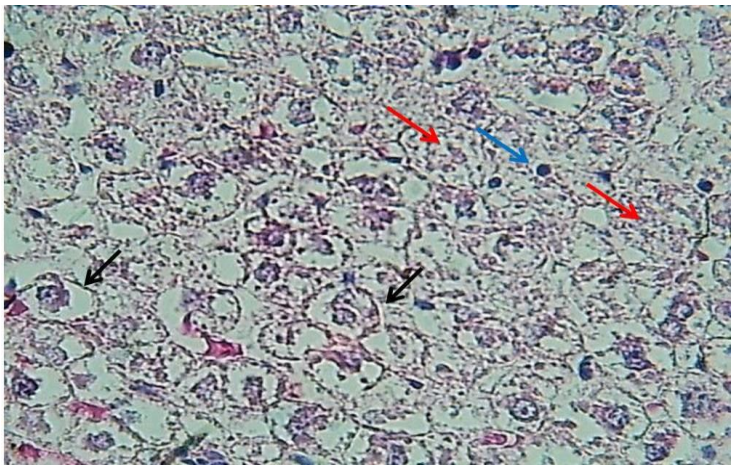

Figure 2: Section of liver (3.5mg/kg- 15 days) show cellular swelling (Black arrows) with necrosis of hepatocytes (Red arrows) with mild infiltration of kupfer cells (Blue arrow) H&E stain.400x.

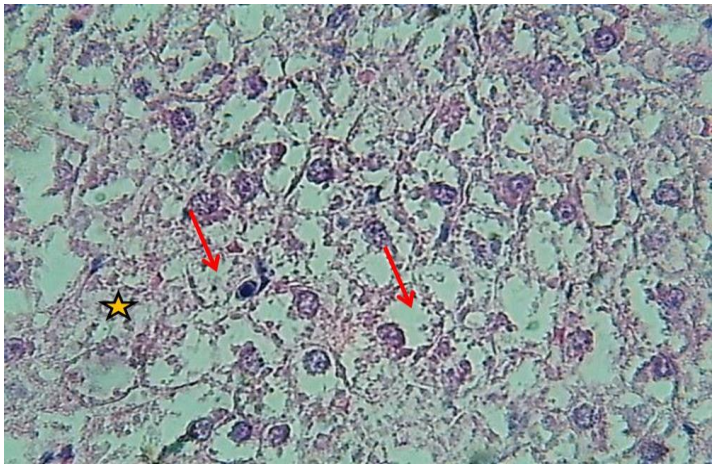

Figure 3: section of liver (7mg/kg- 15 days) shows: sever generalize cellular swelling (Red arrows) with necrosis of hepatocytes (Asterisk). H&E stain.400x.

Exposed group for 30 days.

At this period, the transmission of the damage caused by the pesticide that showed sever dilation with congestion of central vein and hypertrophy of hepatocytes with disarrangement of hepatic cords in section of liver (3.5mg/kg). Group with (7mg/ kg) showed dilation with congestion of sinusoids and mild granular degeneration of hepatocytes. While Section of liver (10.5mg/kg) showed marked sever cellular swelling of hepatocytes with sever necrosis Figure (4,5).

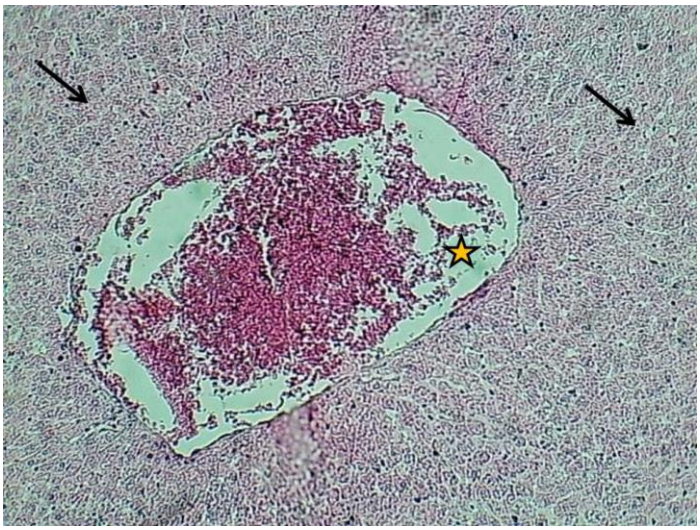

Figure 4: section of liver (3.5mg/kg- 30 days) shows: sever dilation with congestion of central vein (Asterisk) & hypertrophy of hepatocytes with disarrangement of hepatic cords (Arrows). H&E stain.100x.

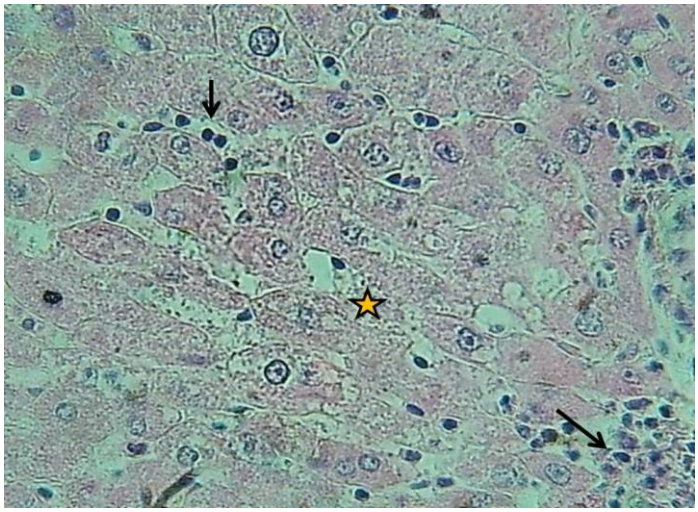

Figure 5: section of liver (3.5mg/kg- 30 days) show hyper atrophy of hepatocytes with mild vacular degeneration and necrosis of others (Asterisk) moderate infiltration of MNCs (Red arrows). H&E stain.400x.

Exposed group for 45 days

All the results shown in the previous two periods and this one indicate that the histopathological changes increase with increasing both the concentration and the duration of exposure. In this period, it was found that all groups exposed to the three concentrations (3.5, 7.0, 10.5 mg/kg) had an increased state of dilation with congestion especially in the sinusoidal and central vein, with an increase in necrosis of hepatocytes and increase of the Kupffer cells inside the sinusoids (Figure 6,7).

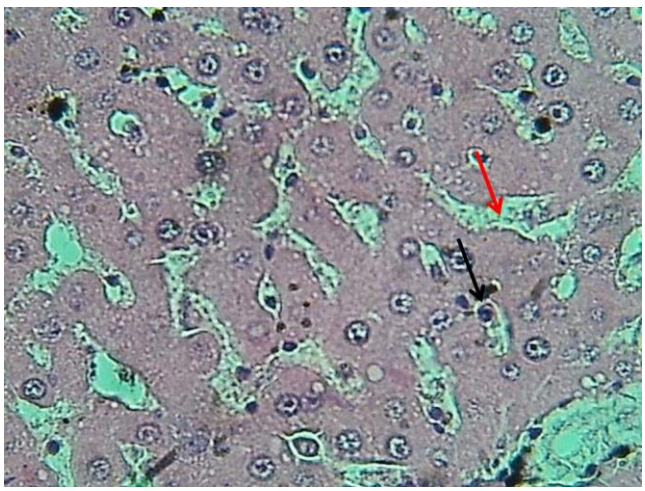

Figure 6: section of liver (3.5mg/kg - 45 days) shows: sinusoidal dilation with congestion (Red arrow) mild infiltration of kupffer cells (Black arrow) H&E stain.400x

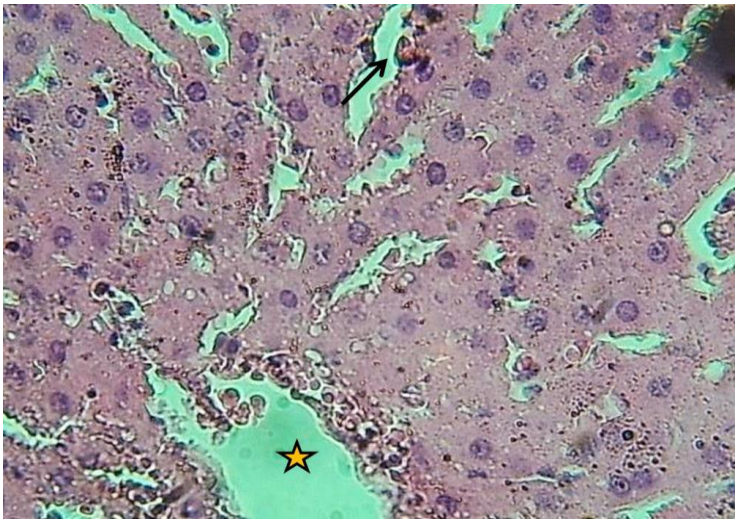

Figure 7: section of liver (7mg/kg- 45 days) shows: mild dilation with congestion of central vein & dilation with congestion of sinusoid (arrows). H&E stain.400x.

DISCUSSION

Biochemical parameters are of paramount importance for assessing many dysfunctions and pathologies, thus making it possible to assess the possible toxic effect of insecticides on the physiological functions of organisms .Liver damage is known as a result of cellular changes in the tissues and alteration in activities of enzymes in liver serum (18; 19). Many studies showed that exposure to these insecticides induces metabolic disturbances such as increased levels of liver enzymes like aspartate amino transferase (AST), alkaline phosphatase (ALP) (20), which is frequently attributed to the toxic metabolic effects of insecticides (21). These enzymes are very sensitive to any xenobiotic that enter the body by the routes of exposure and alteration in their levels consider as a marker of liver injury (22). The hepatic injury is accompanied by hepatic inflammation or necrosis (death of cells). When the hepatic injury is produced, the hepatocytic enzymes are released into the circulation (23).

The present study indicated that the male rats in the endosulfan-treated groups showed increases in AST and ALP levels in the serum of male rats compared to control group. The findings agree with Hatipoglu et al (24) in that the treatment of endosulfan causes an increases in ALP and AST levels in male New Zealand White rabbits (NZW) exposed to (0, 0.75, 1.5, or 3) mg endosulfan/kg. The findings of current study align with prior research highlighting the toxicological effects of Endosulfan on various biological functions such as Abd-Alkazem and Rabee (25) they demonstrated similar results, showing that Endosulfan significantly altered biochemical parameters in white mice, which increased AST levels in treated groups. This result indicates systemic toxicity, particularly on the liver, consistent with Endosulfan's known effects on metabolic organs. Choudhary et al. (26) observed both hepato- and nephrotoxicity of endosulfan in rats. Oral administration of 10 mg/kg/day endosulfan for 2 and 4 weeks caused an increase in ALP activity in numerous organs

combined with cellular damage. Other studies clarify that the elevation in ALP activities may indicates the irritation of the non-specific tissue and usually suggested a toxic effect on the liver with bile duct alterations (27; 28; 16). It is important to note that the elevated activity of serum AST, ALP recorded in the study may be due to tissue of liver enzyme loss. This has been confirmed by hepatocellular damage in the endosulfan dose treated animals.

In accordance with the histopathological changes, the liver is a well-known target organ of the toxic effect regarding its function in the biotransformation and excretion of xenobiotics; therefore, it can be used as a toxicity index for various toxic materials [29,**30**]. The current study has demonstrated that endosulfan has a long-term impact on rats'liver the livers showed several notable alterations, such as mild diltation and congestion progressed to severe necrosis, cellular swelling, infiltration of kupffer cells, degeneration and disarrangement of hepatic cords at higher doses and prolonged exposure. These lesions may arise from the toxic effects of Endosulfan, which disturbs the liver's detoxification mechanisms and induces an inflammatory response comparable with control groups. In present study, Histopathological analysis of liver tissue revealed a hepatic congestion, these findings are in line with those reported by Raj et al (31) when studied the combination of endosulfan and cypermethrin in wistar rats at 207.50 mg/kg bw**.** Additionally, Asma Nazir [32] reported similar liver damage elevations, highlighting the pesticide's potential to cause congestion and diltation in central veins and sinusoids in rat liver produced by a low toxic dose (2.5mg/kg) of endosulfan. The necrosis condition seen in this study is in line with what Sabiha Khan [33] found after subjecting albino rats to the pesticide endosulfan at different doses. Also the present study concur with Kumar et al. [34], who discovered that Endosulfan+OTA combination exposure causes to necrosis in hepatic cells of male rats.

Friday Effiong Uboh et al. (35) absorved Exposure to 5 mg/kg body weight of endosulfan lead to distorted the architectural structure of the liver parankyma as well as necrosis in the liver cells in male albino Wistar rats, this result is consistent with our current sudy. Following pesticide Edosulfan administration, toxic compounds are transported by the blood to various organs including the liver and kidney where they may eventually cause harmful effects (36). The hepatotoxic effects observed in this study are consistent with the work of Hatipoglu et al. (24), who demonstrated that Endosulfan induces hepatic granular degeneration and mononuclear inflammatory cell infiltrations in white rabbit at different doses. Histopathological changes of liver tissue in recent study revealed cellular swelling, these finding were in agreement with Asma Nazir (32) who observed Endosulfan toxicity in male rats at (2.5mg/kg).

The current findings demonstrate notable histological and enzymatic changes in the livers of male rats exposed to Endosulfan alone or in combination with another pesticide. These studies emphasize the hepatotoxic effects of pesticide exposure,

leading to comparable liver damage, as indicated by the increased levels of liver enzymes (AST, and ALP) observed in our research. There is evidence of the relationship between oxidative stress markers and histopathological changes in the liver of animals exposed to pesticides (37). In liver, Endosulfan biologically transformate by cytochrome P450 oxygenase system that produces the highly reactive free radicals which are in turn quenched by the antioxidant systems (38, 39).

Endosulfan was proven to induce free radicals synthesis, such as superoxide radicals (O2 −) and subsequently generate hydrogen peroxide (H2O2) and hydroxyl radicals, highly reactive and harmful to cells (40). The excessive production of these compounds leads to infiltration and activation of macrophages, neutrophils and lymphocytes, increasing the presence of oxidizing agents such as nitric oxide (41). Infiltration of leucocytes caused by endosulfan is usually related to oxidative burst during of inflammation. This process is defined as a rapid and excessive cell release of reactive oxygen species (42). These agents are known to amplify oxidative damage and induce tissue dysfunction (43; 44).

CONCOLUTION

the result of current study demonstrates that a sublethal doses of endosulfan caused oxidative stress in liver. Oxidative stress was shown to be increase in oxygen free radical production. thus, that lead to be alteration in histology and function of liver male albino rat.

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