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N- Acetyl Cysteine Versus Hesperidin as a Prophylactic Agent for Lambda - Cyhalothrin Induced Hepatotoxicity in Adult Male Albino Rats: Histological and Immunohistochemical Study

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ABSTRACT

Introduction: Lambda Cyhalothrin (LCT) is greatly used to manage a wide variety of pests present in farming and in home procedures.

Aim: The current work was intended to demonstrate structural and functional alterations within the liver subsequent to long-standing exposure to LCT. Protective effect of Hesperidin and N- acetylcysteine was also investigated.

Material and methods: 40 adult male albino rats were used in this experiment, and they were split into four equal groups: control, LCT group, rats were received LCT at a dose 61.2mg/kg b. wt. per day by oral gavage for 28 days. LCT + Hesperidin group, rats were given the same dose of LCT with simultaneous oral dosage of HSP at a dose of 100mg/kg b. wt., LCT + N-Acetyl cysteine group at which rats were received the same dose of LCT as the previous 2 groups with simultaneous oral administration of N-acetyl cysteine 150 mg/kg b. wt. The liver underwent a number of biochemical, histological, and immunohistochemical analysis.

Results: LCT induced oxidative stress which leads to liver damage (increases MDA / decreases GSH). LCT caused degeneration of hepatocytes and increases inflammatory cells, this is followed by rise in liver markers (AST and ALT). While concurrent administration of Hesperidin and N- Acetyl cysteine during LCT exposure period preserved the architecture of the liver, prevents its damage, reduced oxidative stress and normalized liver function tests.

Conclusion: Administration of N –Acetyl cysteine during exposure to the insecticide LCT has a protective effect on the liver more than Hesperidin.

Keywords: Liver, Lambda- Cyhalothrin , N-Acetyl Cysteine, Hesperidin.

INTRODUCTION

Lambda-cyhalothrin (LCT) is an artificial pyrethroid which has wide-ranging insecticidal and acaricidal properties; it is usually used in applications where it is able to control insects and pest invasions[(1)].

The liver is a crucial component of the body's metabolic process, converting a variety of nutrients into proteins and secreting bile. It possess a chief purifying role by converting and removing toxins via hepatocyte-facilitated enzymatic mechanisms [(2)].

Metabolism of (LCT) occurs quickly in liver by oxidative and cleavage of ester leading to production of ROS [(3)]. Lipid peroxidation is a result of these ROS' direct interactions with cellular biomolecules leading to damage of DNA and protein oxidation [(4)].

Hesperidin (HSP) is an active flavonoid present plentifully in citrus types as lemon, blood orange, orange and lime. It possesses antioxidant antiviral, anti-inflammatory, analgesic, and anticarcinogenic effect [(5)]. The precursor of the amino acid L-cysteine is N-acetyl cysteine (NAC). It is well recognised that L-cysteine is essential for the production and replenishment of reduced-glutathione (GSH). As one of the most potent antioxidant molecules, GSH can guard tissues from the damaging impacts of ROS under oxidative stress situations [(6)].

The goal of the current study was to demonstrate the potential safeguarding impact of Hesperidin and N-acetylcysteine against Lambda cyhalothrin effect on liver.

MATERIAL AND METHODS

Animals & experimental plan

Forty male mature albino rats 2 months old and weighed 180 - 200 grams were used in this study. We get the rats from the laboratory animals' section of the Veterinary Medicine Faculty at Zagazig University, Egypt. Underneath environmental laboratory condition at temperature 20± 2°C. Rats were retained in plastic cages to escape any metallic contact. Water and typical diet were permitted. The rats were split into four equal groups, each with ten rats after a week of lodging.

- **Group I (Control group):** Three separate groups of ten rats were created:
- **Group Ia:** four rats were given regular diet.
- **Group Ib:** three rats were oral gavaged with distilled water daily.
- **Group Ic :** three rats were given 1% carboxymethylcellulose (CMC) through oral gavage daily.
- **Group II (Lambda-cyhalothrin treated rats) (LCT):** LCT was administered to the rats every day at a dose 61.2mg/kg body weight (1/10 of LD_{50}) through oral gavage after dilution with distilled water for 28 days [(7)].
- **Group III (LCT + Hesperidin):** Rats were given LCT orally as in group II with simultaneous oral administration of HSP for 28 days at a dose 100mg/kg b. wt. daily [(8)].
- **Group IV (LCT + N-Acetylcysteine)**: Rats were received 150mg/kg b. wt. of N-acetyl

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cysteine and 61.2 mg/kg b. wt. of LCT via oral gavage daily for 28 days [9] .

Ethical Consent:

The ethics committee of the Faculty of Medicine at Benha University, Egypt, provided the principles and rules for all the experimental operations. Complied with the US National Institutes of Health's Guide for the care and use of Laboratory Animals. (NIH Publication No. 39/12.062022).

Reagents

Lambda-cyhalothrin (LCT), obtained from MAGICO GROUP for Agrochemical Pharmaceutical, El Obour City, Egypt with commercial name LAMBADA MAGIC 5% . Each litre contains 50gm of Lambda-cyhalothrin and it was diluted in distilled water.

Hesperidin (HSP) obtained from Company for Chemicals Sigma-Aldrich (St. Louis, MO, USA). Hesperidin disintegrated in 1% carboxy methyl cellulose (CMC) which also gotten from Company for Chemicals Sigma-Aldrich (St. Louis, MO, USA).

N-acetylcysteine gained from Sedico Pharmaceuticals Co., Egypt, in the form of (600mg as effervescent instant). It was dissolved in distilled water before being administered.

Sample blood & tissue collection:

1- At the finish of the experiment, the animals were retained without eating all over the night and then they were given intraperitoneal (50mg/kg) of thiopental in the morning. The abdominal cavity was opened longitudinally after fixation of the animals on the dissecting table.
2- collection of blood samples through using a needle stuck in the heart, and then centrifuged at 600g for a period of 10 minutes.
3- Tissues of the liver were crushed using liquid nitrogen to create tissue homogenates.

- **Biochemical analysis:**
- **Liver function parameters:** (AST) and (ALT) were tested [10]. The alkaline medium's sodium p-nitrophenyl phosphate was used as a substrate to quantify ALP activity. [11].
- **Oxidative stress parameters in the liver tissues:**
- **Glutathione (GSH)**: its concentrations are given as mg per gram of tissue [12].
- **Malondialdehyde (MDA):** Its levels were represented as nanomole per mg protein [13].
- **Histological examinations**:

Liver tissues were handled to obtain paraffin slices with a thickness of 5µm after being fixed in 10% formalin for a day. Following deparaffinization, rehydration, and Masson trichrome staining, the sections were processed to show the collagen fibres and hematoxylin eosin to analyse the overall histological structure [14].

Immunohistochemical examination

iNOS:

A polyclonal rabbit anti-iNoS antibody (1:1oo, Abcam, USA) was then applied to the 5-µm liver sections. Then kept at room temperature for 15 minutes before removing. The sections were then counterstained with hematoxylin [15].

Caspase-3:

Slices 5-µm liver tissue were incubated with diluted anti-caspase-3 antibodies from a particular rabbit overnight at 4°C. The pieces were cleaned in purified water and counterstained with hematoxylin [15].

Morphometrical study

Using the image analysis computer system Leica Qwin 500, serial sections stained with iNOS and Caspase-3 were morphometrically examined to reveal the area percent of brown color and also Masson's trichrome stained sections to reveal area percent of collagen fibres at (x 200) magnification. These procedures were carried out in 5 non-overlying fields with 5 distinct sections from 5 different rats in each group.

Statistical analysis

The presentation of all data was mean± standard deviation. The information obtained from the biochemical records and the image analyzer was exposed to (SPSS program; version 20.0 for windows, SPSS Inc., Chicago,IL). Using one Way ANOVA for statistical analysis. The (P) value of ≤ 0.05 was used to determine if the results were significant.

RESULTS

Biochemical results:

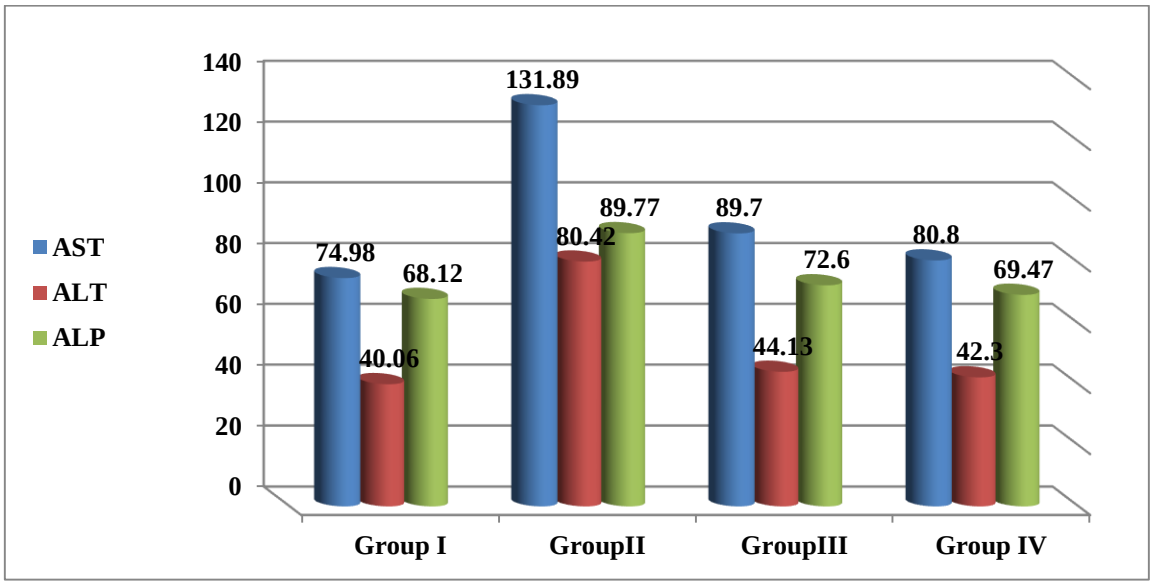
Average serum levels of AST, ALT, and ALP were noticeably higher in group(II) compared to group(I) (P≤0.05). In the group (III) and (IV) they were significantly decreased compared to group (II) (P≤ 0.05) **(Table 1 & Histogram 1).**

The mean tissue MDA level was increased significantly in group (II) compared to group(I) (P≤ 0.05), while in group (III) & (IV) these levels were significantly decreased in comparing with group (II) (P≤ 0.05), **(Table 2 & Histogram 2).**

The mean tissue GSH level was decreased significantly in group (II) compared to group(I) (P ≤ 0.05), while in group (III) and (IV) these levels were significantly increased in comparing with group (II) (P ≤ 0.05), **(Table 2 & Histogram 2).**

Table (1): showing mean values of AST, ALT & ALP IU/ L ± SD in the 4 groups

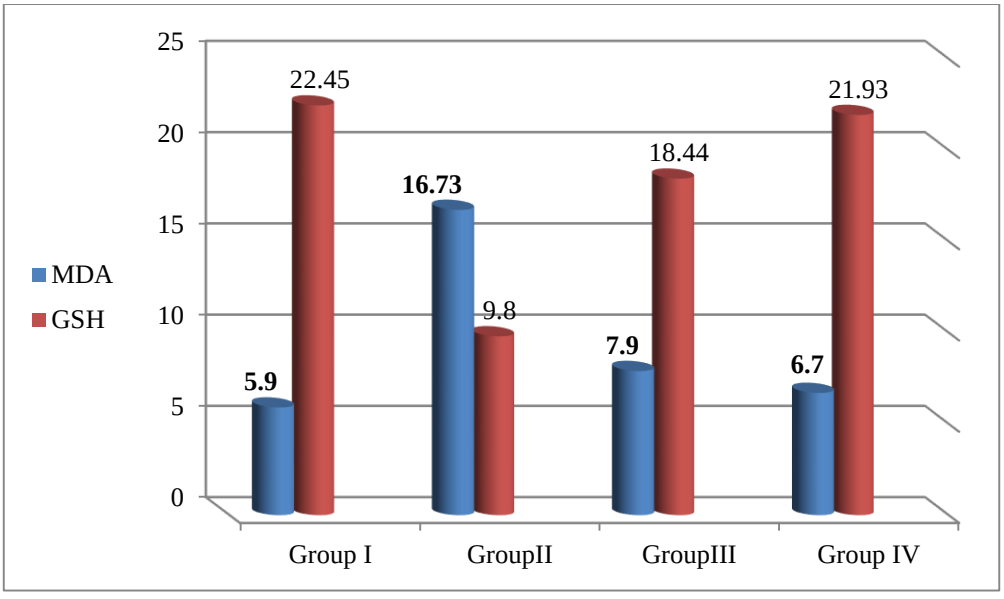
Mean ± SD	GroupI	Group II	Group III	Group IV
AST IU/L	74.98 ± 8.8	131.89 ± 2.3	89.7 ± 1.3	80.8 ± 1.2
ALT IU/L	40.06 ± 3.1	80.42 ± 0.93	44.13 ± 2.5	42.3 ± 1.8
ALP IU/L	68.12 ± 2.1	89.77 ± 1.4	72.6 ± 0.8	69.47 ± 2.3
Significance ≤ 0.05	With group II	With groups I, III & IV	With group II	With group II



Histogram (1): showing mean values of AST, ALT & ALP IU/ L in all groups.

Table (2): Displaying mean values of MDA nmol/ mg protein and GSH mg /gm tissue ± SD in the 4 groups

Mean ± SD	Group I	Group II	Group III	Group IV
MDA nmol/mg protein	5.9± 3	16.73± 2.1	7.9± 3.3	6.7± 3.7
GSH mg/gm tissue	22.45± 2.1	9.8± 1.5	18.44± 0.9	21.93± 1.2
Significance ≤ 0.05	With groupII	With gr0ups I, III & IV	With groupII	With groupII



Histogram (2): viewing mean values of MDA nmol/ mg protein and GSH mg/gm tissue in the 4 groups

Histological Examination:

H & E stain: Group I showed regular polygonal hepatic lobules. Within the centre of the hepatic lobule appeared hepatocyte cords extend outward from the central vein, and blood sinusoids are situated in between the cords. Hepatocytes have acidophilic cytoplasm with vesicular nucleus, some are binucleated (Fig.1A). Bile duct, a branch of the hepatic artery, and a branch of the portal vein made up the portal triad. (Fig.2A). Group II showed hepatocytes with vacuolated cytoplasm and degenerated nuclei, some liver cells showed pyknotic nuclei. Dilated congested blood sinusoids with accumulation of inflammatory cellular infiltrate in between the hepatocytes (Figs. 1B & 1C). Portal area appeared with periportal fibrosis, inflammatory cellular infiltrate, dilated c0ngested p0rtal vein, thickened wall of hepatic artery and hyperplasia of bile duct (Figs. 2B & 2C). GroupIII showed hepatocytes nearly similar to control group but still there is dilated blood sinusoids and some pyknotic nuclei (Fig.1D). Portal area showed decreased inflammatory cellular infiltrate and the periportal fibrosis but still there is congestion of hepatic artery and blood sinusoids (Fig. 2D). Group IV appeared nearly similar to group I with marked improvement when compared to group II, where hepatocytes showed acidophilic cytoplasm with vesicular nucleus and non-dilated sinusoids (Fig.1E). Portal area showed decreased inflammatory cellular infiltrate and periportal fibrosis (Fig. 2E).

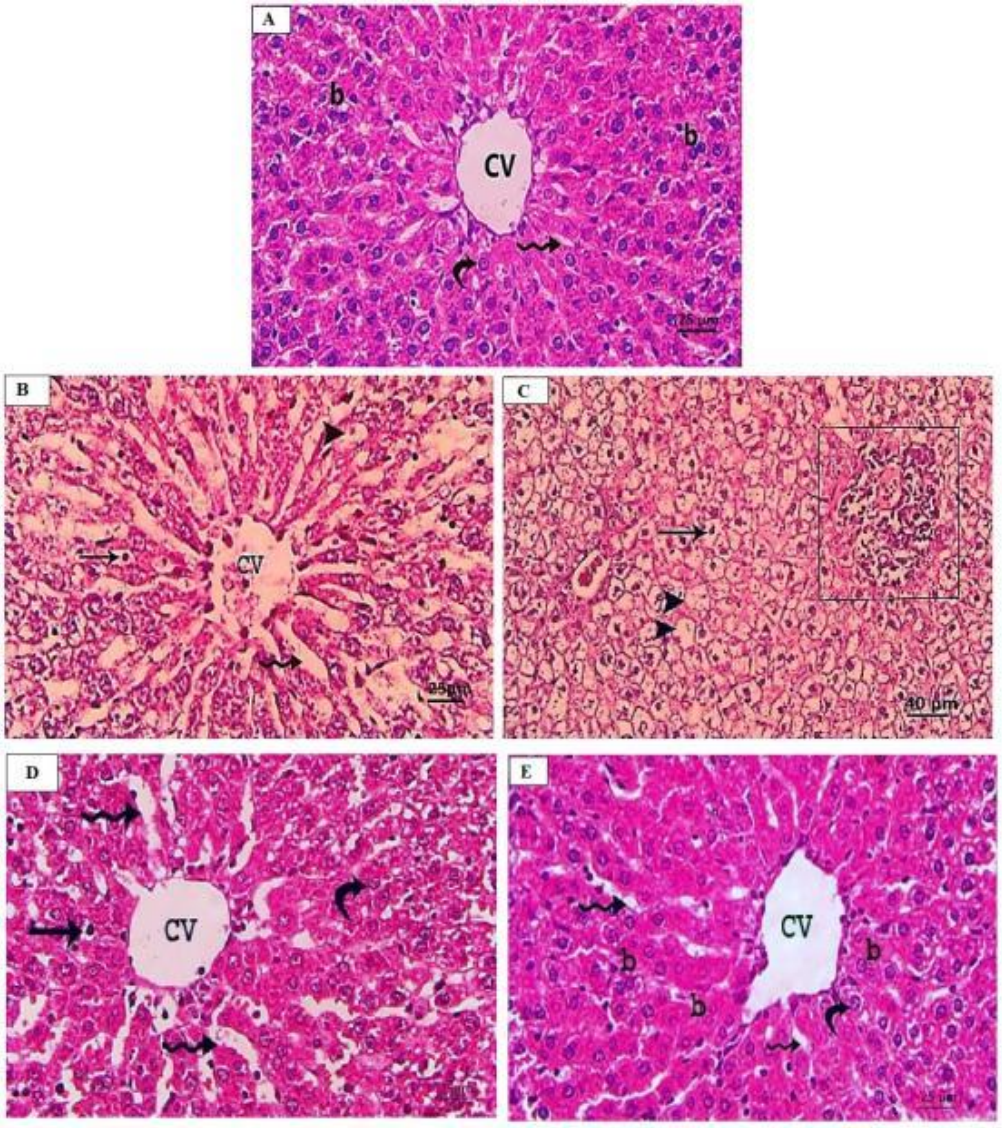


Fig. (1): Photomicrographs of liver sections of **(A): Group I** displaying central vein (CV), hepatocytes with acidophilic cytoplasm and vesicular nucleus (curved arrow), some hepatocytes are binucleated (b). Hepatocytes are separated by sinusoids (wavy arrow) **(H&E X 400). (B): Group II** presenting dilated central vein (CV), some hepatocytes with vacuolated cytoplasm and degenerated nucleus (arrowhead) and other hepatocytes with pyknotic nuclei (arrow), also dilated sinusoids with congestion (wavy arrow) were noticed **(H&E X 400). (C): Group II** demonstrating some liver cells with vacuolated cytoplasm and degenerated nucleus (arrow head) and other hepatocytes with pyknotic nuclei (arrow), infiltration with inflammatory cells (rectangle) **(H&E X 200) (D): Group III** showing central vein (CV), hepatocytes with acidophilic cytoplasm and vesicular nuclei (curved arrow) while few hepatocytes with pyknotic nucleus (arrow), dilated sinusoids (wavy arrow). **(H&E X 400). (E): Group IV** showing central vein (CV), hepatocytes with vesicular nucleus and acidophilic cytoplasm (curved arrow), Some hepatocytes are binucleated (b). Hepatocytes are separated by sinusoids (wavy arrow) **(H&E X 400).**

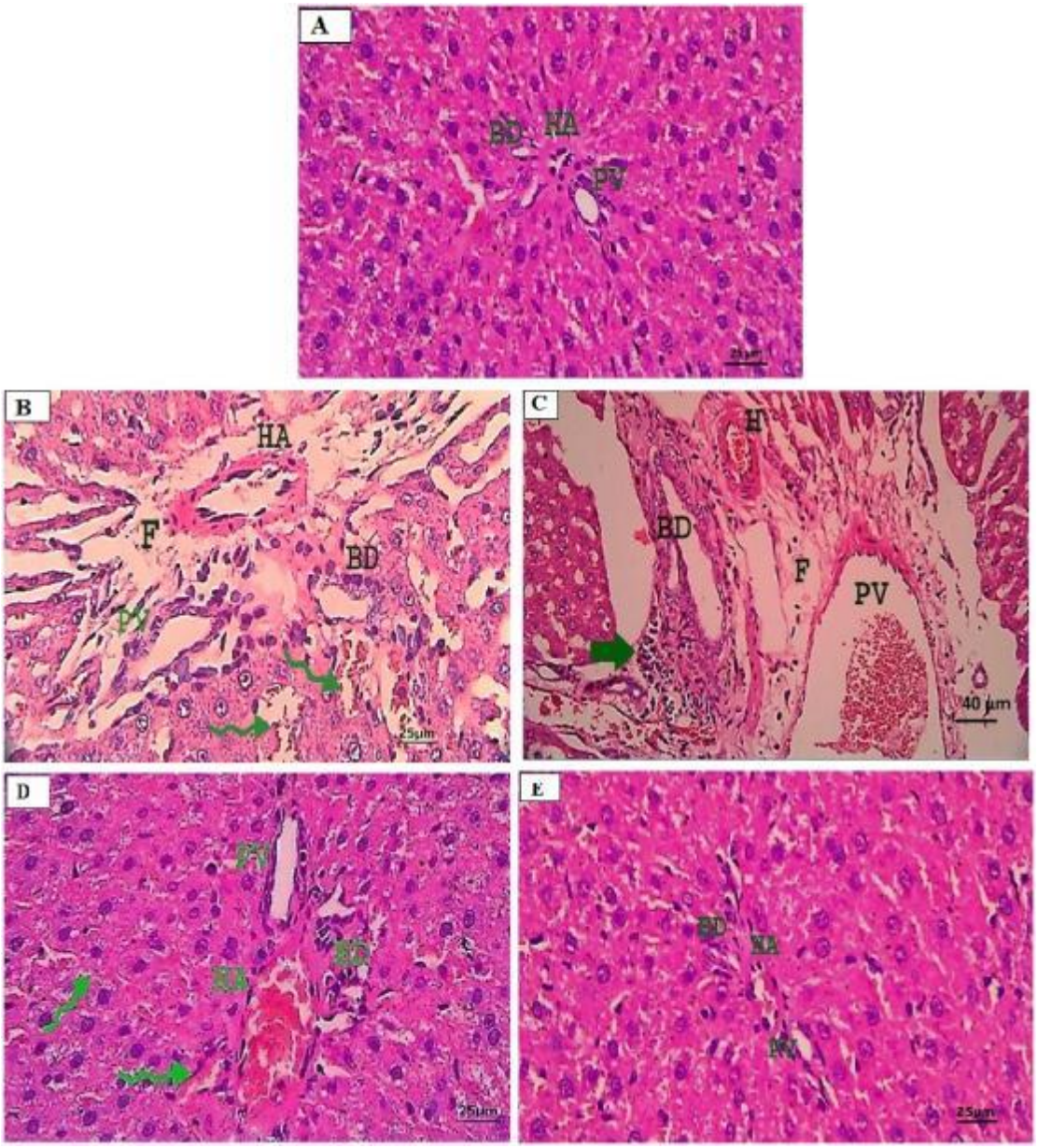


Fig. (2): Photomicrographs of rat liver of **(A)**: **Group I** displaying hepatic artery (HA), portal vein (PV), and bile duct (BD) in the portal region. **(H&E X 400)**. **(B)**: **Group II** presenting hepatic artery with thick hyalinised wall (HA), bile duct with thick wall (BD) and portal vein (PV) with periportal fibrosis (F), dilated sinusoids with congestion (wavy arrow). **(H&E X 400). (C)**: **Group II** showing, hepatic artery with thick hyalinised wall (HA), bile duct hyperplasia (BD), dilated congested portal vein (PV) with periportal fibrosis (F) and inflammatory cellular infiltrate (green arrow) **(H&E X 200). (D)**: **Group III** viewing dilated congested hepatic artery (HA) , Portal vein (PV) ,Bile duct (BD) and dilated sinusoids (Wavy arrow). **(H&E X 400). (E)**: **Group IV** presenting portal area nearly similar to control with hepatic artery (HA), portal vein (PV) and bile duct (BD). **(H&E X 400).**

Masson trichrome stain:

Group I showed very little amount of collagen fibres in between liver cells (Fig. 3A). Group II showed increased amount of collagen fibres in between hepatocytes and around the portal triad (Fig. 3B). Group III showed minimal amount of collagen fibres (Fig. 3C). Very minimal amounts of collagen fibres were seen in group IV (Fig. 3D).

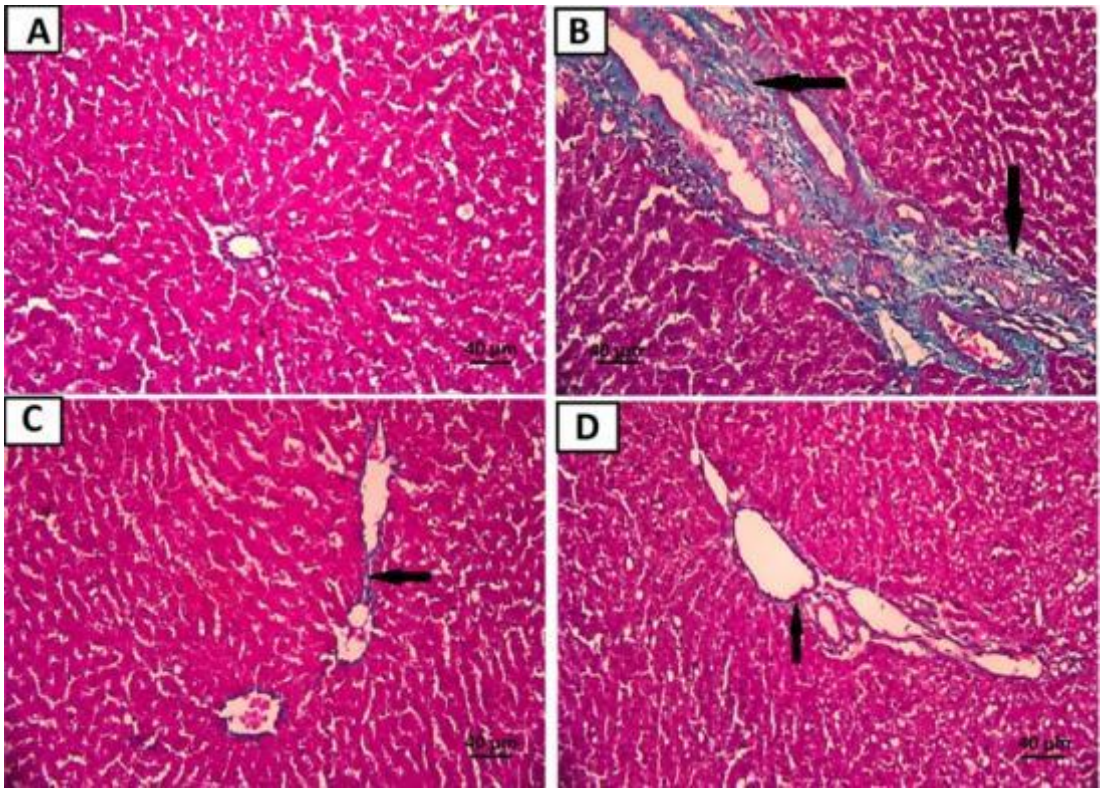


Fig. (3) : (A): Photomicrograph control group's rat liver displaying very minimal amount of collagen fibres between hepatocytes and around portal triad. **(B):** photomicrograph of liver section of Lambda Cyhalothrin treated adult rat viewing increased collagen fibre content in-between hepatocytes and around portal triad (Black arrow). **(C):** photomicrograph of liver section of Lambda Cyhalothrin + Hesperidin cured adult rats presenting few amounts of collagen fibres in-between hepatocytes and around portal triad (black arrows). **(D)**: photomicrograph of liver section of Lambda Cyhalothrin + N Acetyl Cysteine treated adult rats showing very little collagen fibres in-between hepatocytes and around portal triad (black arrow). **(Masson's trichrom X 200).**

Immuno-stained results

iNOs:

The control group revealed negative expression for iNOS (Fig. 4A). Group II revealed positive immuno-reaction for iNOS in the cytoplasm of hepatocytes and around portal triad (Fig. 4B). Group III exhibited weak immuno-reaction for iNOS (Fig. 4C). Group IV showed a very weak immuno-reaction for iNOS (Fig. 4D).

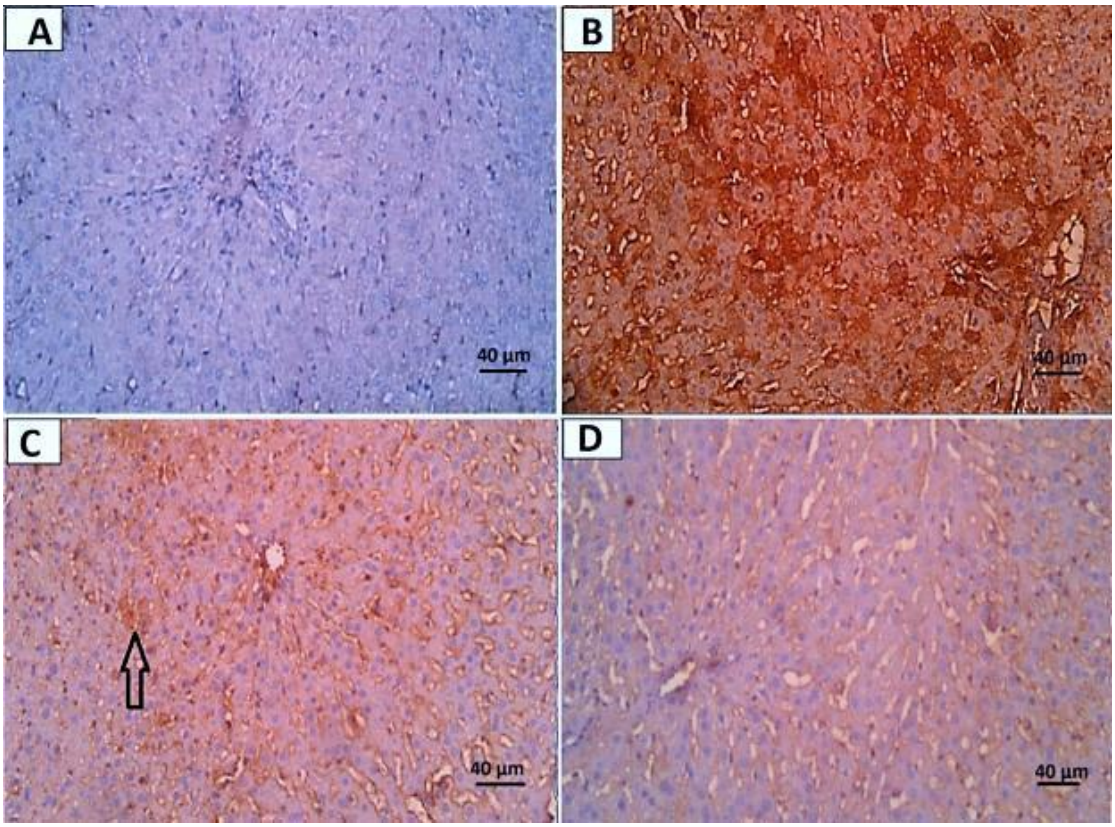


Fig. (4) - photomicrographs of liver section of rat of **(A) : Group I** displaying negative immuno-reaction for iNOS in the hepatocytes cytoplasm. **(B): Group II** viewing positive immunoreaction for iNOS in the hepatocytes cytoplasm. **(C): Group III** showing positive immuno-reaction for iNOS in the cytoplasm of some hepatocytes (arrow). **(D): Group IV** showing very weak immunoreaction for iNOS in the cytoplasm of very few hepatocytes nearly similar to control group **(iNOS immunostaining with counter stain hematoxylin X 200).**

Caspase 3:

Group I revealed negative expression for Caspase 3 (Fig. 5A). Group II revealed strong positive immuno-reaction for Caspase3 in the cytoplasm 0f hepatocytes and around central vein (Fig.5B). Group III revealed weak Caspase 3 immuno-reaction (Fig. 5C). Group IV showed very weak Caspase 3immuno-reaction (Fig. 5D).

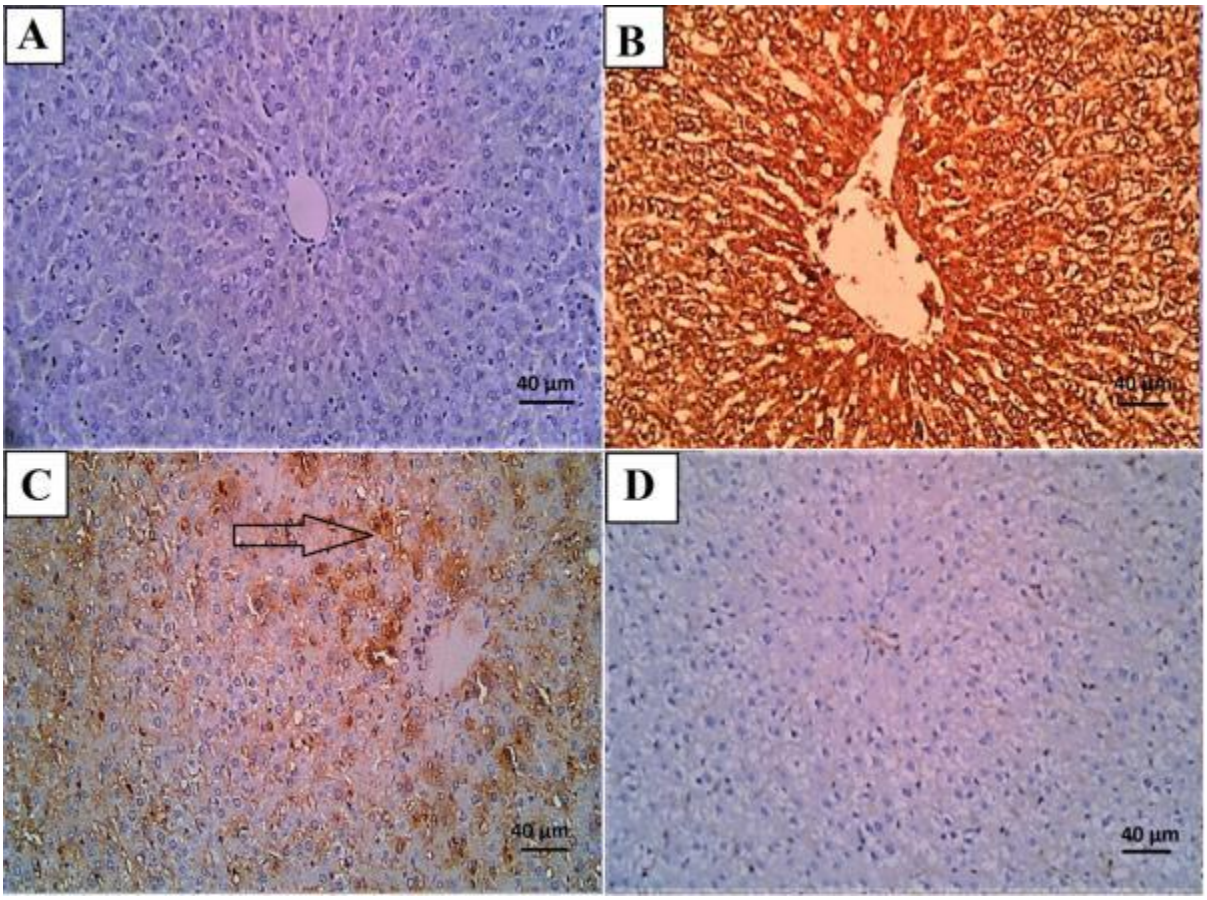


Fig. (5) - Photomicrographs of a rat's liver sections **(A): Group I** displaying negative immuno-reaction for Caspase3 in the hepatocytes cytoplasm. **(B): Group II** presenting positive immuno-reaction for Caspase 3 in the hepatocytes cytoplasm. **(C): Group III** showing positive immuno-reaction for Caspase3 in the cytoplasm of some hepatocytes (arrow). **(D): Group IV** showing very weak immuno-reaction for Caspase 3 in the cytoplasm of very few hepatocytes nearly similar to control group **(Caspase 3 immunostaining with counter stain hematoxylin X 200).**

Morphometric Results:

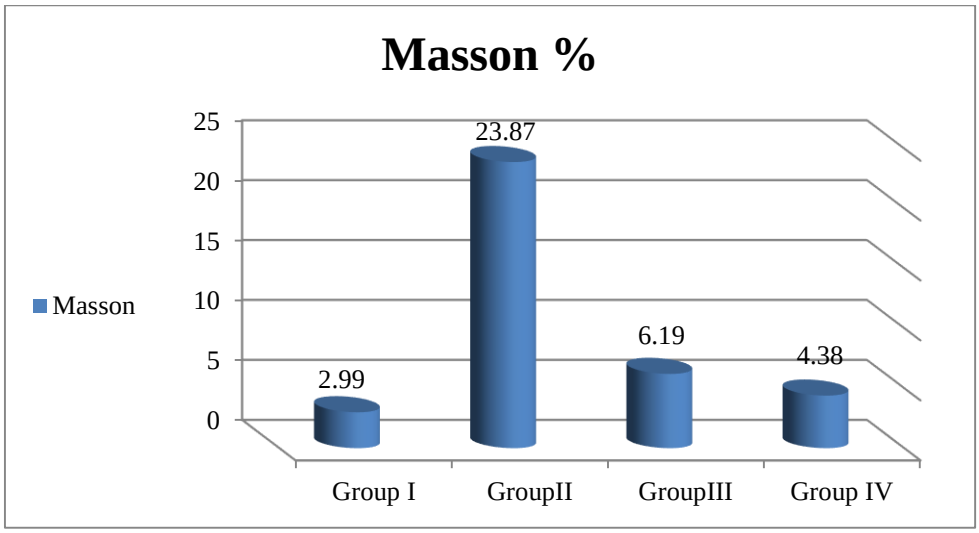
Table (3) and histogram (3) revealed that the average area% of collagen deposition in group(II) exhibited significant rise compared to group (I). While in group(III) and (IV) the area percent of collagen deposition exhibited significant decline, in comparison to group(II) ($P<0.05$).

In table (4) and histogram (4), the mean area percent of iNOs immuno-expression that iNOS immunoreactivity in group(II) exhibited significant rise, compared to group(I). Groups (III) and (IV) demonstrated significant decline, in comparison to group(II) ($P<0.05$).

Table (5) and histogram (5) revealed that the area percent of Caspase 3 immunoreactivity in group(II) exhibited significant rise, compared to group(I). While in group(III) and (IV) the area percent of Caspase 3 immunoreactivity exhibited significant decline, in comparison to group(II) ($P<0.05$).

Table (3): displaying the area's average value percent of collagen fibers deposition ± SD in all groups

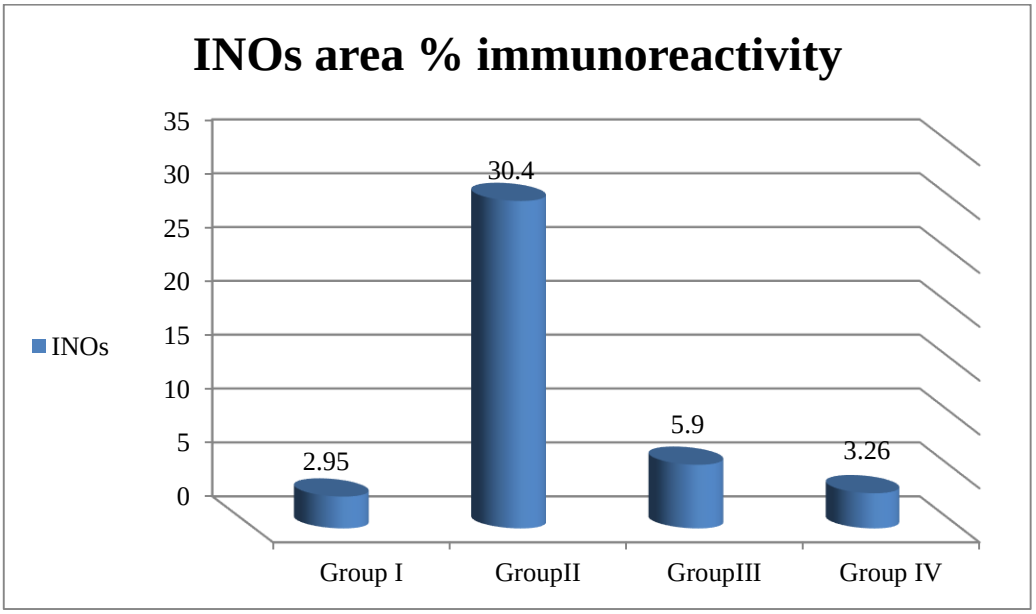
Mean % ±SD	**Group I**	**Group II**	**Group III**	**Group IV**
Masson	2.99 ± 1.99	23.87± 1.44	6.19 ± 2.9	4.38 ± 1.8
Significance ≤ 0.05	With group II	With groups I,III & IV	With group II	With group II



Histogram (3): Showing the mean values of area percent of deposition of collagen fibres in each group.

Table (4): Displaying the averages of the four groups' area percents of iNOS immune expression together with standard deviations

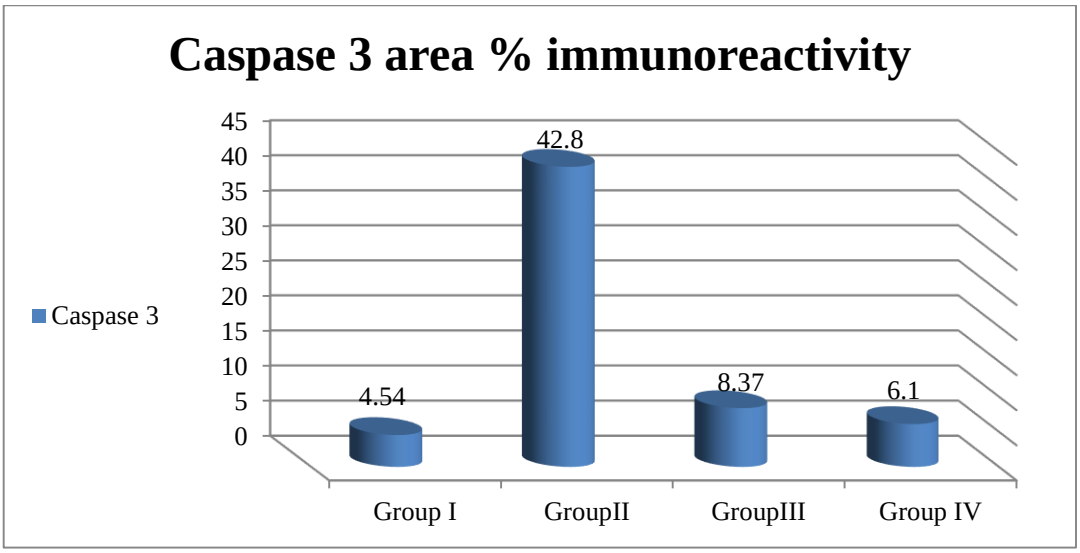
Mean % ± SD	**Group I**	**Group II**	**Group III**	**Group IV**
INOs	2.95 ± 1.36	30.4± 9.5	5.9 ± 1.2	3.26 ± 1.5
Significance ≤ 0.05	With group II	With groups I, III & IV	With group II	With group II



Histogram (4): Showing the average percentage of iNOS in each group.

Table (5): Displaying average values of area percent of Caspase 3 immunoexpression ± SD in each group.

Mean % ± SD	**Group I**	**Group II**	**Group III**	**Group IV**
Caspase 3	4.54 ± 0.58	42.8± 3.5	8.37 ± 0.96	6.1 ± 0.2
Significance ≤ 0.05	With group II	With groups I, III & IV	With group II	With groups II



Histogram (5): Showing the mean values of area percent of Caspase 3 immunoexpression in all groups.

DISCUSSION

LCT has been shown to be harmful to mammals' livers according to **Sankar *et al.*** [16].

This present experiment was planned to assess the role of Hesperidin and N-acetyl cysteine in eliminating LCT hepatotoxicity.

In our study the biochemical assay revealed that the mean values of AST, ALT, ALP and MDA were significantly higher in group (II) compared to group (I) ($P \leq 0.05$). In group(III) and group (IV) they were significantly decreased compared to group (II) ($P \leq 0.05$). The mean GSH level was reduced significantly in LCT group in comparing with control group, whereas in group (III) and (IV) the mean level of GSH were significantly increased in comparing with group (II). This was supported by the work of **Waheed *et al*.** who mentioned that LCT group showed much higher levels of ALT, AST, and ALP were caused by enhanced cell membrane permeability or disruption within the hepatocytes, which allowed these enzymes to seep into the blood [17]. Also **Bhushan *et al*.** reported that the increase in serum ALP concentration have been caused by the destruction of hepatic tissue. Due to the toxicity of pesticides, parenchymal cells in the liver degenerate, allowing ALT, AST, and ALP to leak into the blood stream and create elevated levels [18]. Also **Ben Abdallah *et al*.** revealed that LCT treatment causes male rats to have lower levels of GSH [19].

Hesperidin-treated rats presented a considerable decrease in the levels of AST, ALT, and MDA. However, level of GSH as well as the overall antioxidant capacity were increased [20].

N-acetyl Cysteine has been demonstrated to reduce free radical damage and restore organ dysfunction in animal exposed to pesticides [21]**.** The key factor influencing GSH function in conjugation with produced ROS is its thiol group content. So, supplementing with N-acetylcysteine offers an alternate supply of thiol group and different routes for ROS-conjugation, which aids to replenish depleted GSH levels in the cells [22].

In this experiment H&E-stained liver sections 0f group(II) showed hepatocytes with degenerated nuclei and vacuolated cytoplasm, certain hepatocytes showed pyknotic nuclei. Also, dilated sinusoids with congestion; periportal fibrosis, inflammatory cellular infiltrate, dilated congested portal vein and bile duct hyperplasia were recorded.This goes in line with **Fadina *et al***. who found that liver sections taken from rats intoxicated with LCT revealed infiltration with mononuclear leukocytic, congestion of portal vein with thickening in the wall, with bile duct proliferation. In addition to oedema and hyperemic sinusoids. Hepatocytes exhibited a variety of degenerative alterations, including vacuolar degeneration, pyknosis, hypereosinophilic cytoplasm and karyolysis [23].

Group (III) in our study showed hepatocytes nearly similar to group (I) but with dilated blood sinusoids, and the portal area showed moderate improvement. This goes with **Küçükler *et al***. who verified that administration of HSP 100 with Chlorpyrifos for one month apparently reduced the necrotic and degenerative alterations and infiltration of inflammatory cells was incredibly low [8].

In this work group (IV)`s liver structure appeared nearly similar to control group with marked improvement when compared to group (II). This was clarified by **Presnell *et al*.** who reported that treatment with N-acetylcysteine dramatically lowers the proportion of necrotic tissue in the liver, leading to cytoarchitecture restoration and reduced inflammation. This hepatic enhancement is accompanying to a drop in ALT/AST levels [24].

In this study, increased amount of collagen fibres in between hepatocytes and also around the portal triad in the Masson's-stained liver sections of group (II) while there was minimal amount of connective tissue in

between hepatocytes in group (III). Very minimal amounts of collagen fibres were seen in group (IV). Some authors revealed collagen fibres accumulation in liver tissue of rats intoxicated with LCT [(7)]. Through control of the production of fibrous scar tissue, and anti-inflammatory and antioxidant potentials, hesperidin may be a viable preventative drug against liver fibrosis [(25)].

Abdo *et al.* [(26)] revealed that portal triad and surroundings collagen fibres appeared nearly normal in rats received N-acetyl cysteine.

In our study, liver sections of group (II) revealed positive immuno-reaction for iNOS and Caspase 3 in the hepatocytes' cytoplasm and close to the central vein, This in line with **El-Bialy *et al.*** who proved that insecticides caused oxidative stress in rats, which enhanced iNOS protein expression in hepatic tissue [(27)]. Additionally, **Duzguner & Erdogan** revealed that pesticide intoxication of animals boosted iNOS synthesis and activity as well as caspase-3 gene expression [(28)].

Liver sections of group (III) in this experiment revealed weak immuno-reaction for iNOS and Caspase 3 in a few hepatocytes' cytoplasm. This in agreement with **Küçükler *et al***. who reported that administration of Hespiridin to rats exposed to Chlorpyrifos, drastically decreased amounts of caspase-3, as Hesperidin lowers elevated hepatocyte apoptosis [(8)]. Hesperidin's anti-inflammatory and antioxidant efficiency may be directly due to its antiapoptotic effects [(29)]. Also **He *et al.*** proved that Hesperetin, an active metabolite of hesperidin, dramatically prevented apoptosis by lowering the cleaved-caspase 3 expression [(30)].

In our study liver sections of group (IV) showed very weak immuno-reaction for iNOS and Caspase 3. This agrees with **Allam *et al***. who confirmed that N-Acetyl Cysteine prevented the tissue damage caused by Deltamethrin by reversing the apoptotic reactions that the chemical caused in hepatic tissue [(31)]. **Samuni *et al.*** detected that the anti-inflammatory and immunomodulatory properties of N-acetyl cysteine are what make it protective against liver immunotoxicity and inflammation [(32)].

CONCLUSION

Administration of N –Acetyl cysteine during exposure to the insecticide LCT has a protective effect on the liver more than Hesperidin.

Conflict of interest: The authors say they have no competing interests.

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REFERENCES

1. **Fetoui H, Feki A, Salah G *et al*. (2015):** Exposure to lambda-cyhalothrin, a synthetic pyrethroid,increases reactive oxygen species production and induces genotoxicity in rat peripheral blood. Toxicol. Ind Health , **31**: 433-441.
2. **Lu L, Shi J, Li Q *et al.* (2014):** Effects of liver diseases on drug metabolizing enzymes: implications for drug fate alterations and nano-therapeutic openings. Curr. Med. Chem., 21:2522–2541.
3. **Sankar P, Telang A, Manimaran A (2012):** Protective effect of curcumin on cypermethrin-induced oxidative stress in Wistar rats. Exp. Toxicol. Pathol., 64: 487-493.
4. **Romero A, Ramos E, Castellano V *et al*. (2012):** Cytotoxicity induced by deltamethrin and its metabolites in SH-SY5Y cells can be differentially prevented by selected antioxidants. Toxicol. In Vitro., 26: 823–830.
5. **Ciftci O, Ozcan C, Kamisli O *et al*. (2015):** Hesperidin, a Citrus flavonoid, has the ameliorative effects against experimental autoimmune encephalomyelitis (EAE) in a C57BL/J6 mouse model. Neurochem. Res., 40(6): 1111–1120.
6. **Mokhtari V, Afsharian P, Shahhoseini M *et al.* (2017):** A review on various uses of N-acetyl cysteine. Cell J.,19: 11-17.
7. **Abdul-Hamid M, Mohamed H, Abd El-Twab S *et al.* (2020):** Histological, ultrastructural, and biochemical study on the possible role of Panax ginseng in ameliorating liver injury induced by Lambda cyhalotherin. Beni-Suef Univ. J. Basic Appl. Sci., 9: 52-70.
8. **Küçükler S, Çomaklı S, Özdemir S *et al.* (2021**): Hesperidin protects against the chlorpyrifos-induced chronic hepato-renal toxicity in rats associated with oxidative stress, inflammation, apoptosis, autophagy, and up-regulation of PARP-1/VEGF. Environ. Toxicol., 36(8):1600-1617.
9. **Younes A, Autifi M , Attia H (2020):** Protective Effect of N-Acetyl Cysteine in Acrylamide-Induced Hepatotoxicity in Albino Rats. Al-Azhar International Medical Journal, 1(10): 43-49.
10. **Monteiro M, Xavier A, Oliveira F *et al.* (2016) :** Apoptosis induced by a low-carbohydrate and high-protein diet in rat livers. World J. Gastroenterol., 22(22): 5165-5172.
11. **Bessey O, Lowry O, Brock M (1946):** A method for the rapid determination of alkaline phosphates with five cubic millimeters of serum. J Biol Chem., 164: 321- 329.
12. **Weydert C , Cullen J (2010):** Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat. Protoc., 5(1): 51-66.
13. **Oktay K, Schenken R, Nelson J (1995):** Proliferating cell nuclear antigen marks the initiation of follicular growth in the rat. Biol. Reprod., 53(2): 295-301.
14. **Suvarna S, Layton C, Bancroft J (2019):** Bancroft's Theory and Practice of Histological Techniques. 8th edition. Churchill Livingstone, China. Pp. 408-418.
15. **Buchwalow I, Böcker W (2010):** Immunohistochemistry: Basics and Methods. Springer, Heidelberg, Dordecht, London, New York. Pp. 31–39.
16. **Sankar P, Telang A, Manimaran A (2012):** Protective effect of curcumin on cypermethrin-induced oxidative stress in Wistar rats. Exp. Toxicol. Pathol., 64(5):487-493
17. **Waheed R, Bakery H, El-Shawarby R *et al.* (2011):**Toxicity of lambda cyhalotherin on erythrogram , liver and kidney with molorated by vitamin C. Benha Veterinary Medical Journal, 22(2): 238-248

18. **Bhushan B, Saxena P, Saxena N (2013)**: Biochemical and histological changes in rat liver caused by cypermethrin and beta-cyfluthrin. Arh Hig Rada Toksikol., 64:57–67
19. **Ben Abdallah F, Fetoui H, Zribi N *et al*. (2013):** Quercetin attenuates lambda cyhalothrin -induced reproductive toxicity in male rats. Environ. Toxicol., 28: 673-680.
20. **Rabee A, Bennasir H (2018)**: Hesperidin an antioxidant flavonoid prevents carbon tetrachloride-induced hepatic toxicity in male albino rats. JIPBS., 5:127–132.
21. **Oksay T, Nazıroğlu M, Ergün O *et al*. (2013):** N-acetyl cysteine attenuates diazinon exposure-induced oxidative stress in rat testis. Andrologia ,45:171–177.
22. **Campos R, Shimizu M, Volpini R *et al*. (2012):** N-acetylcysteine prevents pulmonary edema and acute kidney injury in rats with sepsis submitted to mechanical ventilation. Am. J. Physiol. Lung Cell Mol. Physiol., 302(7): L640-L650.
23. **Fadina O, Oshoke F, Fayinminnu O (2017):** Toxicity assessment of synthetic pyrethroids (lambda cyhalothrin) on the liver and kidney organs of male wistar rats. Ann. Res. Rev. Biology., 13(6): 1-7.
24. **Presnell C, Bhatti G, Numan L *et al*. (2013):** Computational insights into the role of glutathione in oxidative stress. Curr Neurovasc Res., 10(2):185-194.
25. **Abdel-Sttar A, Khalaf M, Abo-youssef A *et al*. (2017):** Ameliorative effect of hesperidin on carbon tetrachloride induced liver fibrosis in rats. International Journal of Pharmacy and Pharmaceutical Sciences, 9(7): 45-51.
26. **Abdo F, Ahmed F, Alazouny Z *et al*. (2016) :**Silymarin versus gold nanoparticles efficacy in ameliorating CCL4-induced liver fibrosis in adult male albino rats, A histological and Immunohistochemical study. British j. Sci., 13(2):13-32.
27. **El-Bialy B, Abd Eldaim M, Hassan A *et al.* (2020):** Ginseng aqueous extract ameliorates lambda-cyhalothrin -acetamiprid insecticide mixture for hepatorenal toxicity in rats: Role of oxidative stress-mediated proinflammatory and proapoptotic protein expressions. Environ Toxicol., 35(2):124-135.
28. **Duzguner V, Erdogan S (2012):** Chronic exposure to imidacloprid induces inflammation and oxidative stress in the liver & central nervous system of rats. Pestic Biochem Physiol., 104:58-64.
29. **Abdelaziz R, Abdelazem A, Hashem K *et al*. (2020):** Protective effects of hesperidin against MTX-induced hepatotoxicity in male albino rats. Naunyn Schmiedeberg's Arch Pharmacol., 393(8):1405-1417.
30. **He S, Wang X, Zhong Y *et al.* (2017):** Hesperetin post-treatment prevents rat cardiomyocytes from hypoxia/reoxygenation injury in vitro via activating PI3K/Akt signaling pathway. Biomed Pharmacother., 91:1106–1112.
31. **Allam A, Abdeen A, Devkota H *et al.* (2022) :** N-Acetylcysteine Alleviated the Deltamethrin-Induced Oxidative Cascade and Apoptosis in Liver and Kidney Tissues. Int J Environ Res Public Health, 19(2):638-651.
32. **Samuni Y, Goldstein S, Dean OM *et al.* (2013):** The chemistry and biological activities of N-acetylcysteine. Biochimica et Biophysica Acta., 1830 (8):4117–4129.