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Histological Alterations in Liver Tissues of Male Rabbits Exposed to Cypermethrin Insecticide

Ateeq M.J. Al-Arami*

Department of Life Sciences, Faculty of Science, University of Thamar, Yemen

**Corresponding author*

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A B S T R A C T

Cypermethrin is a sy nthetic pyrethroid insecticide widely used to control various pests in agriculture, public health, and veterinary medicine. However, cypermethrin can cause environmental pollution and pose health risks. The current study was conducted to investigate the histological alterations in the liver of male rabbits following exposure to cypermethrin. The cypermethrin was administered orally at two chronic doses (66.5 mg/kg^{-1}.body.weight and 133 mg/kg^{-1}.body.weight, daily) daily for 21 consecutive days. Histopathological examination of liver tissues from cypermethrin-treated male rabbits revealed multiple alterations, including vascular congestion, ballooning of hepatocytes, degeneration, steatosis, inflammatory cell infiltration, fibrosis, vasodilation, necrosis, amyloidosis, and inflammation. In conclusion, cypermethrin administration at the tested doses induced significant histological changes in the livers of male rabbits.

Introduction

Pesticides can enter the human body through various routes: inhalation via the respiratory system (in the form of volatile compounds or aerosols), ingestion (oral route), and dermal absorption through intact or broken skin (Christos and Ilias, 2011). Long-term exposure to pesticides poses serious health risks and can impair the normal function of various body organs (Hamadache *et al.,* 2016).

Among pesticides, pyrethroids are known to exhibit lower toxicity in mammals. However, they have been reported to disrupt physiological functions and induce pathological changes in animals (Khan *et al.,* 2009). The effects of pyrethroids are often considered immediate, as these compounds are rapidly metabolized within the body (Sayim *et al.,* 2005).

Based on the presence of a cyano group, pyrethroids are classified into two types: Type I and Type II. Cypermethrin, a synthetic Type II cyano-pyrethroid pesticide, is widely used to control agricultural pests, insects, and ectoparasites (Baynes, 2009). It is extensively applied in developing and underdeveloped countries for nearly all forms of pest control (Singh and Saxena, 2001; Atessahin *et al.,* 2005; Eraslan *et al.,* 2008; Bhushan *et al.,* 2013).

Among pyrethroids, cypermethrin is rapidly absorbed in the body and can induce clinical signs of neurotoxicity, including loss of coordination, muscular twitching, and, in severe cases, death due to respiratory failure (Sharaf *et al.,* 2010).

Despite its high efficacy, cypermethrin induces toxicity by disrupting sodium channel function (Narahashi, 1986). Due to its lipophilic nature, the pesticide tends to accumulate in tissues and organs particularly in the central and peripheral nervous systems (Khanna *et al.,* 2002; Laskowski, 2002; Starr *et al.,* 2012). Like many other pesticides, pyrethroids are primarily detoxified and metabolized in the liver and kidneys.

Cypermethrin is widely used by farmers to combat various insects and moths that threaten crops, vegetables, and fruits. However, its residues persist in the environment, exerting harmful effects on a wide range of living organisms, especially wildlife and domesticated animals (Giray *et al.,* 2001).

The main routes of cypermethrin exposure include ingestion, inhalation, and dermal absorption (Noaishi *et al.,* 2013; Côté *et al.,* 2014). Moreover, cypermethrin tends to accumulate within food chains, contributing to its associated toxic effects (Muthuviveganandavel *et al.,* 2011 and Sangha *et al.,* 2013).

Materials and Methods

Chemicals

Generic name: Cypermethrin (10% EC) produced by Vapco company- Amman Jordan

Chemical names: **CAS name**: (RS)-cyano(3-phenoxyphenyl)methyl(1RS)-cis-trans-3-(2, 2 dichloroethenyl)-2, 2-dimethyl-cyclopropane carboxylate

IUPAC name: RS9-Alpha-cyano-3-phenoxybenzyl(1RS)cis-trans-3-(2, 2 dichlorovinyl)-2, 2-dimethyl-cyclopropane carboxylate

Molecular formula: $C_{22}H_{19}O_3NCl_2$

Molecular weight: 416.3

Solubility: In water 0.004 mg/l (pH7), In acetone and chloroform >450 g/l, 20 ^{0}C and; In ethanol 337 g/l, 20 ^{0}C

Melting point: 60-80 ^{0}C
Vapor pressure: 2.3×10^{-4} mPa (20 ^{0}C) (Tomlin, 2006 and 2011).

Ally purchased from authorized dealers of Agrochemical Company, produced by vapco company Amman kingdom Jordan.

Design of Experiment

A total of 18 adult male rabbits (*Oryctolagus cuniculus*) their weights range between (900-1200g). All animals were housed in animal's house in biology department, Faculty of education, University of Thamar, Yemen. The animals were healthy and acclimatized for 14 days prior to the start of the study. Were used in the experiment animals were divided into three groups (each of 6 rabbits). The first group was served as control group, were given normal saline daily by disposable syringe. The second group was given oral dose equivalent to 1/10 LD_{50} (66.5 mg/kg^{-1}.body.weight) of cypermethrin every day (Total of 21 Treatments) while the third group given oral high dose equivalent to 1/5 LD_{50} (133 mg/kg^{-1}.body.weight) of cypermethrin alone every day by disposable syringe (Total of 21 Treatments), cypermethrin was dissolved and diluted to the required doses using distilled water.

The experimental animals were maintained in the animal house on daily observations and well fed by rabbit's chaw under good conditions of ventilation and at room temperature of 25-27°C, relative humidity of 50±150c and a normal photoperiod of 12 hours/day. Animals were given the same quantity of food and water *ad-Libitum* throughout the study.

Histological Alterations

After terminal, all rabbits were necropsied and the liver was removed, washed in normal saline, fixed in 10% formalin for 24 hours. The liver washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax (melting point of 50-56 c), Paraffin sections were cut at 6ᴍ m thicknesses using a rotary microtome, the sections were stained with harris haematoxylin and eosin. Observation were made using a light microscope and photographs were taken with an automatic photo micrographic system. (Jaber and Al-Bakri, 2018; Al - Hamawandy and Al-Bakri, 2020 and Jaber *et al.,* 2020).

Results and Discussion

Histopathological Changes in the Liver

Liver sections from the control group treated with a physiological solution for 21 days showed normal hepatic architecture. The hepatic lobules exhibited a thin-walled central vein, with normal hepatic cords radiating toward the periphery, alternating with hepatic sinusoids (Figure 1).

In contrast, examination of liver cross-sections from rabbits treated with cypermethrin at a concentration of 66.5 mg/ kg^{-1} body weight for 21 days revealed various histopathological alterations. These changes varied in severity and type among the treated animals and can be summarized as follows: steatosis (fatty degeneration), ballooning of hepatocytes, necrosis, congestion, degeneration, fibrosis, vasodilation of blood vessels, and cellular infiltration (Figures 2, 3, 4, 5, 6 and 7)

While treatment with cypermethrin at a concentration of 133 mg/kg^{-1} body weight for 21 days showed an increase in the effects on liver tissue, which were manifested in Congestion of blood vessels, Inflammation, Infiltration, Amyloidosis, Degeneration, Steatosis (Fatty degeneration) and Ballooned Hepatocytes (Figures 8, 9 and 10).

The histopathological changes observed in rabbit liver tissues in our study are in agreement with several previous studies. Giray *et al.,* (2001) reported that cypermethrin induces oxidative stress in the brain and liver of rats, and that this effect can be mitigated by vitamin E or allopurinol.

Similarly, Bhushan *et al.,* (2013) found that histopathological examination of rat liver tissues from treated animals revealed various cellular and lobular abnormalities, including cytoplasmic vacuolization and hepatocyte membrane damage. Our findings are also consistent with those of Abdus Sallam *et al.,* (2020), who reported that cypermethrin toxicity in rabbits caused cytoplasmic vacuolization, hepatocyte membrane damage and necrosis

Furthermore, our study aligns with that of Faten and Abdulhadi (2016), who observed marked blood congestion in the hepatic vein, infiltration of inflammatory cells, sinusoidal dilatation, hemorrhage, distortion of cell arrangement, hepatocyte hypertrophy, cellular degeneration and necrosis, pyknotic nuclei, and cytoplasmic vacuolation in the livers of cypermethrin-treated rats.

Manna *et al.,* (2004) conducted a single oral dose toxicity study of alpha-cypermethrin in rats, which supports our findings. Additionally, Latif *et al.,* (2011) observed that cypermethrin-induced hepato-renal pathological changes in rabbits included condensation of hepatic nuclei and cytoplasmic vacuolation in 50% of the animals initially, later affecting all treated animals. In later stages, these changes became more severe and extensive, showing dose-dependent cellular degeneration in hepatic parenchyma, along with regenerating hepatocytes.

The pathological changes in hepatocytes due to cypermethrin may be attributed to its inhibitory effect on total ATPase activity in the liver, disrupting the active transport of Na^{+}, K^{+}, and Ca^{2+} ions, and subsequently injuring the hepatocytes (Khan *et al.,* 2009).

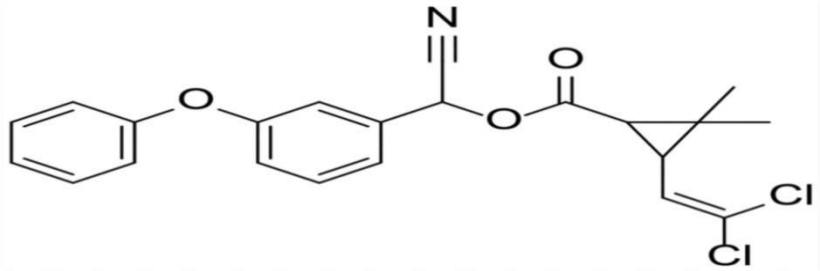
Soliman *et al.,* (2011) also reported that livers of male Wistar rats exposed to cypermethrin showed central vein congestion, cellular infiltration, necrosis, and vacuolation—findings that are confirmed by our current study.

Moreover, Abdou *et al.,* (2012) observed similar degenerative changes in hepatocytes, such as congestion, lymphocytic infiltration, necrosis, and vacuolation. Acute administration of cypermethrin led to significant degeneration in the histological architecture of liver tissues.

In a more recent study, Farzeen and Razia (2022) reported that cypermethrin exposure in rabbits affected blood profiles and redox parameters, in addition to causing histopathological changes such as infiltration, necrosis, vacuolation and degeneration findings consistent with our results. Ahmad and Khan (2011) also demonstrated that administering cypermethrin at doses of 50, 100, and 150 mg/kg produced moderate histological lesions in rabbit liver tissue over time.

Similarly, Yassin and Hadi (2016) reported that dietary cypermethrin at doses of 7.5, 15, and 30 mg/kg body weight caused blood congestion in hepatic veins, sinusoidal dilatation, hepatocyte hypertrophy, cellular degeneration, necrosis, pyknotic nuclei, and cytoplasmic vacuolation in rat liver tissue.

Figure.1 Chemical structure of cypermethrin (Lin *et al.*, 2011)



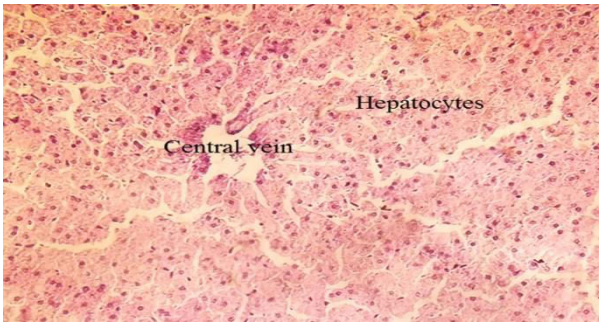


Figure 2. Transverse section (T.S.) from rabbit liver of group 1 (control) treatment with physiological solution for 21 days shows: Normal hepatic architecture with normal parenchymal hepatocytes, normal sinusoidal lumens, outwardly decided hepatic cords round the central vein and Normal hepatocytes with centrally located nuclei (H&E Stain, 100X)

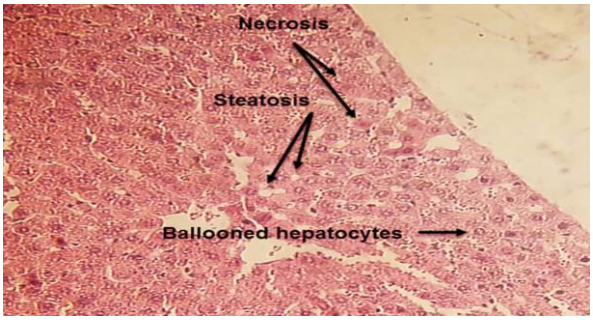


Figure 3. Transverse section (T.S.) from rabbit liver, of group 2, treated with 66.5 mg/ kg-^{1}.body.weight/ day Cypermethrin for 21 day shows: Steatosis, Ballooned Hepatocytes and Necrosis (H&E Stain, 100x).

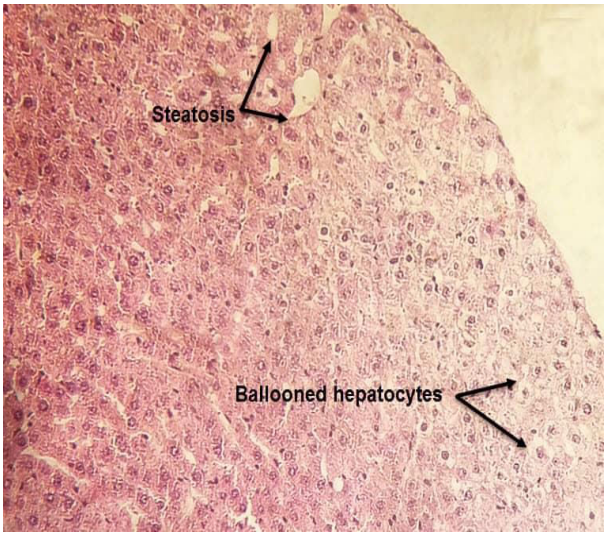


Figure 4. Transverse section (T.S.) from rabbit liver, of group 2, treated with 66.5 mg/ kg-^{1}.body.weight/ day Cypermethrin for 21 day shows: Steatosis and Ballooned Hepatocytes (H&E Stain, 100x).

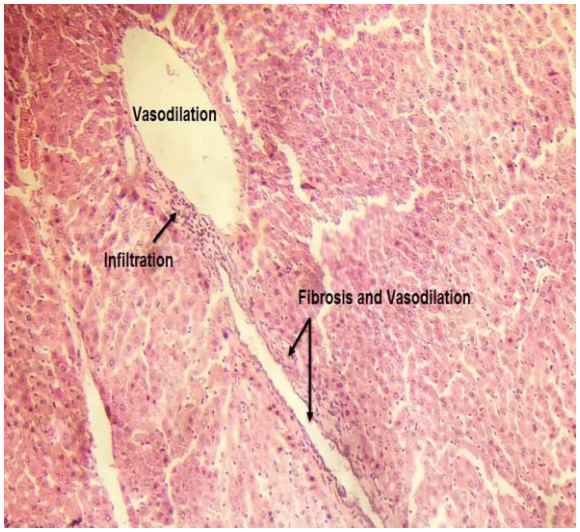


Figure 5. Transverse section (T.S.) from rabbit liver, of group 2, treated with 66.5 mg/ kg-^{1}.body.weight/ day Cypermethrin for 21 day shows: Fibrosis and vasodilation and Infiltration (H&E Stain, 100x).

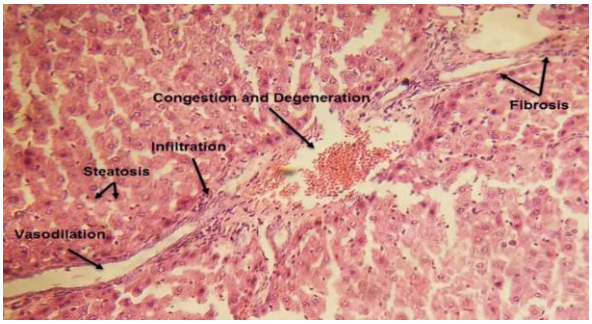


Figure 6. Transverse section (T.S.) from rabbit liver, of group 2, treated with 66.5 mg/ kg-^{1}.body.weight/ day Cypermethrin for 21 day shows: Congestion and Degeneration, Fibrosis and vasodilation, Steatosis and Infiltration (H&E Stain, 400x).

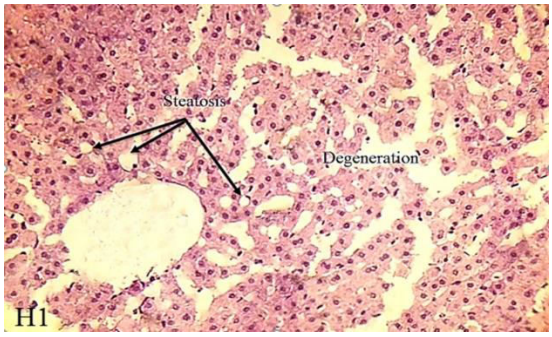


Figure 7. Transverse section (T.S.) from rabbit liver, of group 2, treated with 66.5 mg/ kg-^{1}.body.weight/ day Cypermethrin for 21 day shows: Vasodilation, Congestion and Fibrosis (H&E Stain, 400x).

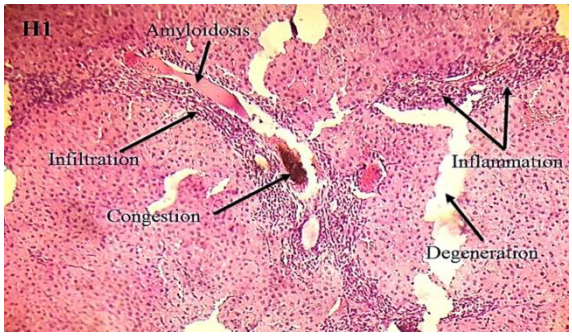


Figure 8. Transverse section (T.S.) from rabbit liver, of group 3, treated with 133 mg/ kg-^{1}.body.weight/ day Cypermethrin for 21 day shows: Congestion, Inflammation, Infiltration, Amyloidosis and Degeneration. (H&E Stain, 400x).

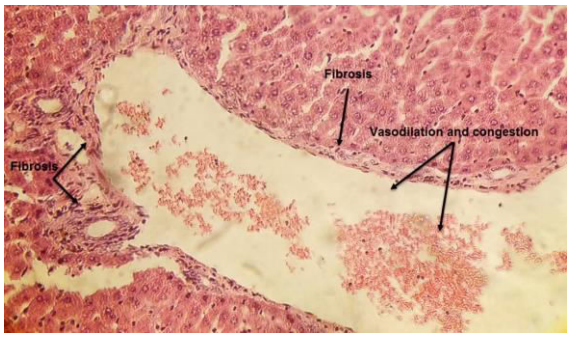


Figure 9. Transverse section (T.S.) from rabbit liver, of group 3, treated with 133 mg/ kg-^{1}.body.weight/ day Cypermethrin for 21 day shows: Degeneration and Steatosis. (H&E Stain, 400x).

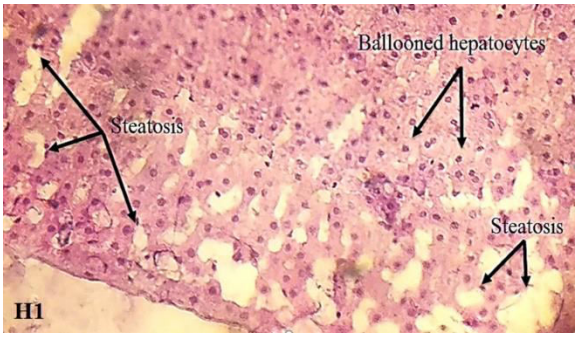


Figure 10. Transverse section (T.S.) from rabbit liver, of group 3, treated with 133 mg/ kg-^{1}.body.weight/ day Cypermethrin for 21 day shows: Ballooned Hepatocytes and Steatosis. (H&E Stain, 400x).

Our findings are further supported by Duliami (2023), who observed signs of central vein and sinusoidal congestion, mild vacuolar degeneration, hepatocyte necrosis, sinusoidal dilation, and focal infiltration of inflammatory cells in the liver. Structural changes in the liver due to cypermethrin toxicity may also result from glycogen depletion and inhibition of ester-hydrolyzing enzymes (Burns & Pastoor, 2018; Alabsy & Alabdaly,

2022). Furthermore, hypoxia and altered protein catabolism may contribute to histopathological changes (Khan *et al.,* 2010).

Saxena *et al.,* (2005) noted that cypermethrin disrupts cell membrane integrity by penetrating the lipid bilayer, leading to potential DNA damage, including instability, helix unraveling, and chromosomal lesions.

In conclusion, our study demonstrates that cypermethrin induces significant histopathological changes in rabbit liver tissue, consistent with numerous previous studies.

These changes include cellular degeneration, necrosis, vacuolization, hepatocyte hypertrophy, inflammatory infiltration, and sinusoidal alterations.

The results support the hypothesis that cypermethrin toxicity disrupts hepatocellular function through oxidative stress, enzyme inhibition, ionic imbalance, and structural damage at the cellular and subcellular levels.

Recommendations

It is recommended to issue and enforce strict laws and regulations governing the use of pesticides in agriculture, due to their potential health risks to both animals and humans.

Author Contributions

Ateeq M.J. Al-Arami: Investigation, formal analysis, writing—original draft.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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