Abstract
Excessive use of pesticides has led to increasing concern about undesirable effects on human health and the environment. Imidacloprid is an internationally used neonicotinoid insecticide due to its significant toxicity to insects. Its residues may reach the food chain, which is important for examining the potentially harmful properties of imidacloprid exposure. The current study aimed to characterize the histopathological effects of imidacloprid on the liver of male rabbits.

The Imidacloprid administration daily at the two chronic oral doses of (45 mg/kg and 90 mg/kg, daily) for 37 days. Treated male rabbits groups for treatment concentrations revealed many histopathological changes in the liver, such as congestion of blood vessels, hemorrhage, inflammation, ballooned hepatocytes, steatosis, infiltration, vasodilatation, necrosis and fibrosis. The study results established that Imidacloprid was significantly worse in specific organs in the digestive system (liver) of male rabbits.

Keywords: Imidacloprid, Histopathology, Liver, Toxicity, Male rabbits.

1. Introduction
Worldwide, the use of pesticides has altered the properties of acute and chronic poisoning, with variable risk effects on human health, from minor effects to death [1]. Internationally, about 3 million cases are described each year that occur due to acute pesticide poisoning. Cut 3 million cases of pesticide poisoning, 2 million are reckless efforts, and the rest are due to work-related or accidental poisoning [2].

The term pesticide includes many compounds that contain insecticides, fungicides,
herbicides, rodenticides, plant growth hormones, and others [3]. Pesticides play a dynamic role in agricultural production; however, due to their toxicity, they also cause particular health concerns for humans and other animals [4-5]. Indeed, pesticide information is a multifaceted compilation and toxicity evidence on active ingredients alone is not satisfactory for assessing the risks of conflicting health effects of commercial pesticides [6].

Imidacloprid was discovered in 1984 by Nihon Bayer Agrochem in Japan by demonstrating new synthetic mixtures with a great affinity for the nicotinic AChRs of insects, with little toxicity to vertebrates [7]. Imidacloprid is an organic compound used as a pesticide [8]. Chemically, it is [1-(6-chloro-3-pyridylmethyl)-nitroimidazolidin-2-ylideneamine] as well as a new neonicotinoid insecticide and is currently the most widely used insecticide in contrast to a broad spectrum of sucking and chewing pests [9-11]. It is used to control sucking insects and some biting insects. It can be useful topically for pets for flea control, as well as for formulations, crops, soil, and seed treatments [12]. Imidacloprid is used to protect seedlings from root and leaf pests, in addition by managing leaves in the late season [13].

Imidacloprid causes near-complete and irreversible blockade of nicotinic acetylcholine receptors (nAChRs) in the central nervous system (CNS) of insects, and these receptors are located within the central nervous system. Globally, imidacloprid is increasingly being sold as an insecticide because it has a high selectivity for insects and appears to be safe for humans [14].
Imidacloprid can enter the body via oral, dermal, and inhalation routes. More than 90% of its excretion occurs within 24 hours, thus very less residual effect [15-16]. Hepatotoxicity is the primary effect observed in the imidacloprid toxicity [17].

The toxicity of imidacloprid refers to the mutagenic and carcinogenic effects in humans and animals [18]. Long-term exposure to imidacloprid leads to genotoxic effects and oxidative stress in rabbits [19-21]. The liver is a significant goal organ for the toxicity of drugs and xenobiotics owed to its joining with the gastrointestinal tract as well as for the singularity and difficulty of its anatomical construction and metabolic functions. It can be considered a target organ of numerous chemicals either in the workplace or at home [22].

Histological techniques are widely used as a potential toxicity marker for different environmental pollutants, including insecticides [23]. The current study was designed to study the histopathological effects of the insecticide Imidacloprid, widely used in some agricultural areas in Yemen, on specific organs of laboratory animals.

2. Materials and Methods

Chemicals

Generic name: Imidacloprid (20% EC)

Trade names: Admire, Acidor, Comodor, Commando (by Arab pesticides & veterinary drugs Mfg. co.), Confidate, Confidor, Gaucho (Gustafson LLC.); Imadate, Marathon (by Olympic Horticultural produces).

Chemical Names: CAS Name: 2-Imidazolidinimine, 1-((6-chloro-3-pyridinyl)methyl)-N-nitro-

IUPAC name: 1-(6-chloro-pyridin-3-yl-methyl)-N-nitrom - dazolidine - 2 ylidniamine

CAS number 138261-41-3 Molecular formula: C_9H_10ClN_5O_2 Molecular weight: 255.66

Solubility: 0.58 gm/L of water at 20°C. Solvable in each of acetone, acetonitrile, dimethylformamide, DMSO and methylene chloride.

Ally purchased from authorized dealers of Agrochemical Company, produced by the kingdom Jordan.

The Median Lethal Dose (LD50) oral dose for male rabbits used in the current research is 450 mg kg was determined based on the toxicology method [26;27] and categorized Imidacloprid as abstemiously hazardous. Imidacloprid was dissolved and diluted to the compulsory dosages by distilled water.

3. Experimental design

A total of eighteen healthy domestic male rabbits (Oryctolagus cuniculus domesticus) of ages range (3-4 months and weight ranges (900-1100 g). All animals were housed in the animal house,
Department of Biology, College of Applied Sciences, Thamar University, Yemen. The animals were healthy and conditioned for 15 days before the start of the study.

The animals were continued in the animal house on daily clarifications they well fed by rabbits under good ventilation conditions of humidity at room temperature and a regular photoperiod of 12 h/day. These animals were given the same amount of ad-Libitum food and water through the study.

The experimental animals were divided into three groups (each group had six rabbits). The first group was the control group, and they were given normal saline daily with a disposable syringe. The second group was treated with an oral dose equal to 1/10 LD50 (45 mg/kg body weight) of Imidacloprid each day however the third group was treated with a high oral amount equal to 1/5 LD50 (90 mg/kg body weight) body weight.) of Imidacloprid alone every day with a disposable syringe the treatment continued for 37 days.

4. Histopathological Studies

After the end of the experiment, all rabbits were dissected. The liver was separated, washed with normal saline, fixed in 10% formalin for 24 hours. The liver was washed in tap water; dehydrated with ascending concentration of ethanol, and cleared in a xylene solution embedded in paraffin wax (melting point of 50-56 ° C). Paraffin sections after that were cut at 6μm thicknesses using a rotary microtome device made in Britain; the sections finally were stained with Harris haematoxylin and eosin stain. The histopathological examination were investigated using an optical compound light microscope and images were captured using an automatic microscopy imaging system [28-30].

5. Results and Discussion

Histopathological Aterations in the liver

The basic structure of normal liver sections showed numerous hepatic lobules. Each of which contains cords of regularly organized hepatocytes enclosing the sinusoidal network. The central vein is positioned in the middle of the lobule. Hepatocytes are polygonal with granular eosinophilic cytoplasm and a central nucleus with one or two nucleoli and fine strands of chromatin. Also, Kupffer cells appeared among the hepatocytes as spindle-shaped cells (Figure 2).

The liver histological sections of group 2 of the treated animals (1/10 LD50 of imidacloprid) appeared liver hemorrhage (Figure-3), Congestion and Ballooned Hepatocytes (Figure-4) Inflammation (Figure- 5) and Steatosis (Figure- 6).
Figure 4. Transverse section (T.S.) from rabbit liver, of group 2, dosage with 45 mg/ kg/ day Imidacloprid for 37 day, shows: congestion and ballooned hepatocytes, (H&E Stain, 20 X).

Figure 5. Transverse section (T.S.) from rabbit liver, of group 2, treated with 45 mg/ kg/ day Imidacloprid for 37 day, shows: inflammation, (H&E Stain, 20 X).

Figure 6. Transverse section (T.S.) from rabbit liver, of group 2, treated with 45 mg/ kg/ day Imidacloprid for 37 day showing Steatosis (H&E Stain, 40X ).

Furthermore, liver histological sections of the group 3(1/5 LD50 of imidacloprid) displayed infiltration (Figure- 7 and 9), foci necrosis (Figure- 8), liver Hemorrhage, Ballooned Hepatocytes and Necrosis (Figure- 8 and 9) and Vasodilation, foci necrosis and Fibrosis (Figure- 10).

Figure 7. Transverse section (T.S.) from rabbit liver, of group 3, treated with 90 mg/ kg/ day Imidacloprid for 37 day, shows: Infiltration (H&E Stain, 20X ).

Figure 8. Transverse section (T.S.) from rabbit liver, of group 3, dosage with 90 mg/ kg/ day Imidacloprid for 37 day, shows: liver hemorrhage, foci necrosis, ballooned hepatocytes and necrosis, (H&E Stain, 20 X).
The histopathological investigation effects in this study confirmed the treated group with the 1/10 of LD50 of Imidacloprid for 37 days caused on by degenerative alterations in the liver with hemorrhage, congestion, ballooned hepatocytes, inflammation and steatosis. Similarly, liver section of rabbits in this group was treated with a high dose (1/5 LD50) of Imidacloprid displayed Infiltration, hemorrhage, ballooned hepatocytes, necrosis, vasodilation, foci necrosis, and fibrosis. The effect at higher doses was more harmful. The liver was a well-recognized target organ for toxicological influence in connection with its utility in biotransformation and elimination of xenobiotics [31].

These results correspond with many authors; [32] observed that Japanese quails treated with 1/50 LD50 of Imidacloprid for 3 and 6 weeks showed degenerative changes in the liver. The previous studies also reported that after three weeks of the recovery period liver revealed the irregular arrangement of hepatocytes with abnormal architecture, large area of necrosis, dilated sinusoidal spaces, large and small areas of degeneration with faintly stained cytoplasmic nuclei. [33] observed that 90-day oral intoxication in female rats with 20 mg/kg/day caused mild focal necrosis of hepatocytes with swollen nuclei in the liver. It was also noted that oral administration of imidacloprid at a dose of 139 mg/kg resulted in hemorrhage and pallor in the kidneys. [17]

Microscopically, the liver tissues of the intoxicated chicken showed congestion, hemorrhage, degeneration of hepatocytes, coagulative necrosis, and mild dilation of the hepatic sinuses. The observations in this study agree with [34] that imidacloprid doses at 80 mg/kg bw/day in an oral dose for 28 days in male rats caused marked dilation, congestion of the central vein, portal vein, sinus spaces, lumen, fatty change and degenerated hepatocytes in the liver.

While ultrathin sections of the liver showed swollen nuclei, different sizes, and different features of mitochondria, disturbed chromatin, and rough endoplasmic reticulum. The companion with [35] observed that oral administration of doses of 25, 50, and 75% LD50 in female albino mice caused deterioration of hepatocytes, dilation of sinuses, arrangement of irregular hepatic cords, leukocyte infiltration, necrosis, and hemorrhage in the liver. The severity of the lesions was more significant with increasing imidacloprid dose.
6. Conclusion

This study concluded that exposing male rabbits to the insecticide Imidacloprid with different concentrations has led to histopathology changes in the rabbit's liver.

The effect has increased with increased dose concentration chronic exposure to this pesticide may be an important factor in the overall vulnerability of these animals to pathogens, which facilitates and accelerates their diseases and the economic productivity of mammalian animals.

References
1. Dawson, A.H.; Eddleston, M.; Senarathna, L.; Mohamed, F.; Gawarammana, I.; Bowe, S.J.; Manuweera, G.; Buckley, N.A. Acute human lethal toxicity of Agricultural pesticides: a prospective cohort study. PLoS Medicine. 2010, 7(10) :1-10. www.plosmedicine.org
2. Singh, B.; Mandal, K. Environmental impact of pesticides belonging to newer chemistry. Integr. Pest Manag. 2013, 152-190.
3. Cope, W.G.; Leidy, R.B.; Hodgson, E. Classes of toxicants: use classes. In: Textbook of Modern Toxicology. 3rd ed, John Wiley and Sons, Inc., New Jersey. 2004, 58-70.
4. Maroni, M.; Colosio, C.; Faioli, A.; Fait, A. Biological monitoring of pesticide exposure: a review. Introd. Toxicol. 2000, 143, 1-118.
5. Bjorling-Poulsen, M.; Andersen, H.R.; Grandjean, P. Potential developmental neurotoxicity of pesticides used in Europe. Env. Health. 2008, 7, 50,1-23.
6. Mansour, S.A.; Mossa, A.H. Comparative effect of some insecticides as technical and formulated on male Rats. J. Egypt. Soc. Toxicol. 2005, 32, 41-54.
7. Kagabu, S. Chloronicotinyl insecticides—discovery, application and future prospective. Rev. Toxicol. 1997, 1, 75-129.
8. California Environmental Protection Agency (Cal EPA). (2006). Risk Characterization Document for Imidacloprid. Available at: http://www.cdpr.ca.gov/docs/risk/rcd/imidacloprid.pdf.
9. Wismer, T. Novel Insecticides. Clinical Veterinary Toxicology; Plumlee, K. H., Ed.; Mosby: St. Louis, MO. 2004, 184-185.
10. Tomlin, D.S. The Pesticide Manual, A. World Compendium, 14edition: British Crop Protection Council: Surry, England, 2006, 598-599.
11. Yeh, I.J.; Lin, T.J.; Hwang, D.Y. Acute multiple organ failure with imidacloprid and alcohol ingestion. Taiwan, Am J Emerg Med. 2010, 28, 2, 255.
12. National Pesticide Information Center (NPIC). Imidacloprid: Technical Fact Sheet. 2010.
13. Morrissey, C.A.; Mineau, p.; Devries, J.H.; Sanchez-Bayo, F.; Liess, M.; Cavallaro, M.C. and Liber, K. Neonicotinoid contamination of global surface waters and associated risk to aquatic vertebrates: a review. Environ Int. 2015, 74, 291-303.
14. Matsuda, K.; Buckingham, S.D.; Kleier, D.; Rauh, J.J.; Grauso, M. and Sattelle, D.B. Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. Trends Pharmacol. Sci. 2001, 2, 11, 573-80.
15. Klein, O.; Karl, W. Methylene-[14 C] Imidacloprid: Metabolism Part of the General Metabolism Study in the Rat. Bayer AG. Leverkusen-Bayerwerk, Germany, Study. 1990, (87264), 51950-0021.
16. Broznić, D.; Marinić, J.; Tota, M.; ČanadiJurešić, G. ;Milin, Č. Kinetic evaluation of imidacloprid degradation in mice organs treated with olive oil polyphenols extract. *Croatica Chemica Acta*. **2008**, *81*, 1, 203-209.
17. Kammon, A.M.; Brar, R.S.; Banga, H.S. ; Sodhi, S.Pathobiochemical studies on hepatotoxicity and nephrotoxicity on exposure to chlorpyrifos and imidacloprid in layerchickens. *Veterinarski Arhiv*. **2010**, *80*, 5, 663-672.
18. Karabay, N.U.; Oguz, M.G.Cytogenetic and genotoxic effects of the insecticides,imidacloprid and methamidophos. *Genet. Mol. Res*. **2005**, *4*, 653–662.
19. Stivaktakis, P.; Kavvalakis, M.;Goutzourelas, N.; Stagos, D.; Tzatzarakis, M.; Kyriakakis, M.; Rezaee, R.; Kouretas, D.; Hayes, W. and Tsatsakis, A.Evaluation of oxidative stress in long-term exposed rabbits to subtoxic levels of imidacloprid. *Toxicol. Lett.* **2014**, *228*-229.
20. Stivaktakis, P.D.; Kavvalakis, M.P.; Tzatzarakis, M.N.; Alegakis, A.K.;Panagiotakis, M.N.; Fragiadaki, P.; Vakonaki, E. ; Ozcagli, E. ; Hayes, W.A.;Rakitskii, V.N. and Tsatsakis, A.M.Long-term exposure of rabbits to imidacloprid as quantified in blood induces genotoxic effect. *Chemosphere*. **2016**, *149*,108–113.
21. Vardavas, A.I .; Ozcagli, E. ; Fragiadaki, P. ; Stivaktakis, P.D. ; Tzatzarakis, M.N. ; Kaloudis, K. ; Tsardi, M. ; Datseri, G. ; Tsiaoussis, J. ; Tsiptsimpikou, C. ; Carvalho, F. and Tsatsakis, A.M.DNA Damage after Long-term Exposure of Rabbits to Imidacloprid and Sodium. *Tungstate*. **2016**, *258*,247–248.
22. Arafa, U.A.; Fujiwara, Y.; Higuchi, K.; Shiba, M.; Uchida ,T.; Watanabe ,T.; Tominaga, K.; Oshitani, N.; Matsumoto, T. and Arakawa, T.No additive effect between Helicobacter pylori infection and portal hypertensive gastropathy on inducible nitric oxide synthase expression in gastric mucosa of cirrhotic patients. *Dig Dis Sci*. **2003**, *48*, 1, 162-168.
23. Bhaiyan, A.S.; Nesa, B. and Nessa, Q.Effects of Sumithion on the Histological Changesof Spotted Murrel, Channapunctatus (Bloch). *Pakist. J. Biol. Sci*. **2001**, *4*, 10, 1288-1290.
24. Kramer, F. and Mencke, N. *Flea Biology and Control: the biology of the cat flea,control and prevention withimidacloprid in small animals*. SpringerVerlag, Berlin,Heidelberg,New York. 2001
25. World Health Organization (WHO). *The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification*, Geneva. IPCS. **2005**
26. Weil, C.S.Tables for convenient calculation of median effective dose (LD 50 or ED50) and instructions in their use. *Biometrics*. **1952**,8, 249.
27. Memon, S.A.; Memon, N.; Mal, B.; Shaikh, S.A. :Shah, M.A.Histopathological changes in the gonads of Male rabbits (*Oryctolagus cuniculus*) on exposure to imidacloprid insecticide. *J. Entomol.Zool. Studies*. **2014**, *2*, 4, 159-163.
28. Jaber, A.T. Nawar R. and Al-Bakri, N.A.Effect of Carbamazepine Drug on Liver Ultrastructure in Female White Mice (Mus musculus). *Int.J.Pharmac.Res*. **2018**, *10*, 4.
29. Al-Hamawandy,H. and Al-Bakri, N.A. Analysis of Fatty Acids of Liver in Embryoadult of Domesticated Chicken Gallus Gallus Domesticus. *J. Global Pharma Technol*. **2020**, *11*, 5, 62-68.
30. Jaber, A.T. Nawar R.; Al-Bakri, Nahla, A. ; Al-Bhadly, A. T.Association Between Carbamazepine Toxicity, Liver Bile Duct Injury, Granuloma and Inflammatory Cells Infiltration in Female Mice. *Indian J. Foren. Med &Toxicol*. **2020**, *14*, 4, 1773-1778.
31. Roganovic, Z. D. ; Jordanova, M. Liver lesions in bleak (AlburnusalburnusalborellaFilippi) collected from some contaminated sites on lake Ohrid. A histopathological evidence. Ekol. Zast. Zivot. Sred. 1998, 6, 11-18.
32. Eissa, O.S. Protective effect of vitamin C and glutathione against the histopathological changes induced by imidacloprid in the liver and testis of Japanese quail. Egypt. J. Hosp. Med. 2004, 16, 39-54.
33. Bhardwaj, S.; Srivastava, M.K.; Kapoor, U. and Srivastava, L.P. A. 90 days oral toxicity of imidacloprid in female rats: morphological, biochemical and histopathological evaluations. Food Chemical Toxicol. 2010, 48, 5, 1185-1190.
34. Soujanya, S.; Lakshman, M.; Kumar, A.A. ; Reddy, A.G. Evaluation of the protective role of vitamin C in imidacloprid-induced hepatotoxicity in male Albino rats. J. Nat. Sci., Biol. Med. 2013, 4, 1, 63-67.
35. Kumar, A. ; Tomar, M. ; Kataria, S.K. Effect of sub-lethal doses of imidacloprid on histological and biochemical parameters in female albino mice. ISOR J Environ Sci T oxicol Food Technol. 2014, 8, 9-15.