Pesticides and Non-alcoholic Fatty Liver Disease

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most common type of chronic liver injury worldwide and its spectrum is inclusive of steatosis, steatohepatitis, cirrhosis, and hepatocellular carcinoma. It has been related to obesity, insulin resistance, diabetes, and other metabolic syndromes. Numerous studies have been conducted to test the association between pesticides and fatty liver disease. Many of pesticides are lipophilic and tend to accumulate in the adipose tissue. The liver is the first-line of defense against potentially harmful xenobiotics through metabolism by cytochrome P450 enzymes. However, if the accumulated concentration of pesticides reaches lethal levels due to high dose or chronic exposure, they can overwhelm the liver detoxification capacity and cause toxicity. The mechanisms of their action in NAFLD development and progression involve insulin resistance, deposition of lipids in the hepatic parenchyma, oxidative stress, altered production of adipokines, which ultimately may leading to steatohepatitis and cirrhosis. Although the pathogenesis of pesticides-associated NAFLD is not yet fully understood, there is increasing elucidation of the mechanisms of progression from steatosis to more advanced liver inflammation and fibrosis. Such knowledge may eventually translate into the development of novel treatment strategies for this increasingly important condition.

Keywords: Non-alcoholic fatty liver disease, Pesticides

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สารเคมีกำจัดศัตรูพืชและโรคไขมันพอกตับที่ไม่ได้เกิดจากแอลกอฮอล์

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บทคัดย่อ

โรคไขมันพอกตับที่ไม่ได้เกิดจากแอลกอฮอล์ เป็นการบาดเจ็บเรื้อรังที่ตับที่พบได้บ่อยที่สุด มีการสะสมไขมันในตับสูง แต่อาจรุนแรงขึ้นเป็นตับอักเสบ โรคตับแข็งและมะเร็งตับ โรคไขมันพอกตับมีความสัมพันธ์กับโรคอ้วน การดื้อต่ออินซูลิน โรคเบาหวานและโรคอ้วนลงพุงอื่นๆ มีการศึกษาจำนวนมากที่แสดงถึงความสัมพันธ์ระหว่างสารเคมีกำจัดศัตรูพืชและโรคไขมันพอกตับ พบว่าสารเคมีกำจัดศัตรูพืชหลายชนิดมีคุณสมบัติชอบไขมันและสะสมในเนื้อเยื่อไขมัน ตับเป็นอวัยวะด่านแรกที่ช่วยป้องกันสารพิษที่อาจเป็นอันตรายโดยผ่านกระบวนการเมตาบอลิซึม แต่อย่างไรก็ตาม ถ้าสารเคมีกำจัดศัตรูพืชสะสมถึงระดับที่เป็นอันตรายเนื่องจากได้รับในปริมาณสูงหรือการสัมผัสเรื้อรัง ก็สามารถทำลายการขับสารพิษของตับและก่อให้เกิดความเป็นพิษต่อตับได้ กลไกการเกิดโรคเกี่ยวข้องกับการดื้อต่ออินซูลิน การสะสมของไขมันในเนื้อเยื่อตับ ภาวะเครียดออกซิเดชั่น การเปลี่ยนแปลงการหลั่งของ adipokines ซึ่งในที่สุดอาจนำไปสู่ตับอักเสบและโรคตับแข็ง แม้ว่าพยาธิกำเนิดของโรคที่เกี่ยวข้องกับสารเคมีกำจัดศัตรูพืชจะยังไม่สามารถอธิบายได้อย่างสมบูรณ์ แต่ปัจจุบันการศึกษาการดำเนินโรคจากการสะสมไขมันในตับที่นำไปสู่การอักเสบของตับและมีพังผืดในตับก็มีเพิ่มมากขึ้น ซึ่งองค์ความรู้นี้อาจจะนำไปสู่การพัฒนากลยุทธ์การรักษาแบบใหม่ได้

คำสำคัญ: โรคไขมันพอกตับที่ไม่ได้เกิดจากแอลกอฮอล์ สารเคมีกำจัดศัตรูพืช

***ผู้รับผิดชอบบทความ**

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Introduction

Liver is an important organ for human health. It is the center of crucial metabolic activities, and represent the first line of defense against toxic compounds. Liver is subjected to several types of insults and diseases, which able to alter its functions[1]. One of the most common cause of abnormal liver function tests and the most prevalent chronic liver diseases worldwide is the non-alcoholic fatty liver disease (NAFLD). NAFLD is a multifactorial disease closely associated with metabolic syndrome, and considered its hepatic manifestation[2]. Its prevalence worldwide is thought to be approximately 20% in the general population and up to 70% in type 2 diabetes mellitus patients[3]. NAFLD involves a wide spectrum of liver pathologies with key stages ranging from simple steatosis to the more aggressive form of non-alcoholic steatohepatitis (NASH), which in turn may lead to fibrosis, cirrhosis, and in some cases, hepatocellular carcinoma[4]. Nonetheless, the underlying mechanisms of liver steatosis are still unclear. Although metabolic derangements have been established as main risk factors for NAFLD, a growing body of evidence supports the idea that pesticides exposure may have an impact on liver diseases, including NAFLD[1].

The term pesticide covers a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and others[5]. Pesticides are used almost everywhere, not only in agricultural fields, but also in homes, parks, buildings, forests, and roads. Ideally, a pesticide must be lethal to the targeted pests, but not to non-target species. Unfortunately, the rampant use of these chemicals, under the adage, “if little is good, a lot more will be better” has played havoc with human and other life forms[5]. Pesticides can contaminate soil, water, turf, and other vegetation. Several studies have demonstrated that many pesticides have been associated with liver disease and elevated levels of liver enzymes aminotransferases[6]. However, the mechanisms by which these compounds potentiate liver disease and damage are poorly studied. Therefore, this review article will focus on the role of pesticides in NAFLD and the mechanisms of their action in the potentiation and progression of steatohepatitis.

Non-alcoholic fatty liver disease (NAFLD)

NAFLD refers to the accumulation of fat, mainly triglycerides, in the liver (hepatic steatosis) either on imaging or on liver histology and there are no causes of secondary hepatic fat accumulation such as significant alcohol consumption, steatogenic medication usage, and hereditary disorders. NAFLD is further categorized histologically into non-alcoholic fatty liver (NAFL) and

NASH. NAFL is defined as the presence of hepatic steatosis with no or minimal inflammation and no fibrosis. NASH is defined as the presence of hepatic steatosis with inflammation, ballooned hepatocytes (hepatocyte injury), and/or fibrosis (which may progress to cirrhosis)[3, 7].

The initial theory for the pathogenesis of NAFLD was the 'two-hit' theory[8]. The first hit is hepatic fat accumulation or steatosis. This can be brought about by excessive consumption of hyper-caloric diets, and increases susceptibility of the liver to injury mediated by the second hit. The second hit results in hepatic and systemic inflammation which are accompanied by increased levels of inflammatory cytokines/ adipokines, mitochondrial dysfunction and oxidative stress and causes steatohepatitis. This may then progress to fibrosis and cirrhosis[9]. Moreover, industrial chemicals and toxic pollutants including pesticides are the other second hit factors leading to the term "toxicant-associated steatohepatitis" (TASH)[6]. Widely used pesticides promote the occurrence of hepatic steatosis[1]. One potential mechanism underlying this pathological process involves the activation of the nuclear receptor called pregnane X receptor (PXR). PXR is mainly expressed in liver and intestine. It is involved in the integrity of the endocrine system and modulates the metabolism and elimination of xeno- and endobiotics[10]. The activation of PXR leads to increased hepatocellular lipid content, through increased intestinal lipid uptake and triglycerides synthesis, due to the up-regulation of genes involved in fatty acid uptake and mobilization [i.e., fatty acid translocase (CD36) and fatty acid binding protein 2 (FABP2)], and to the activation of the sterol responsive element binding protein (SREBP). Elevation of hepatic lipid content leads to an increase of the AMP/ATP ratio[11]. Pesticides may also induce steatosis, obesity and insulin resistance by causing mitochondrial dysfunction[1]. NAFLD is increasingly diagnosed worldwide in a large proportions of the population across all age ranges, which will continue for the foreseeable future[12]. Because of its strong association with obesity, diabetes, arterial hypertension and hypertriglyceridaemia. This is universally considered as the hepatic manifestation of the metabolic syndrome and insulin resistance which is regarded as its key pathophysiological hallmark[13].

Pesticide

According to the US Environmental Protection Agency, a pesticide is defined as "any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest". The term "pesticide" is not only refer to insecticides, but it actually applies to all the substances

used to control pests. Types of well-known pesticides classified by use including insecticides, herbicides, rodenticides, and fungicides[14]. Pesticides share similar chemical properties or act on the pest in the same way, and often grouped into families. A pesticide product may have active ingredients from more than one chemical family. Common families of pesticides include organophosphates, organochlorines, carbamates, and pyrethroids[15]. Their formulations contain both active and inert ingredients. Active ingredients act to control the pests and inert ingredients play key roles in pesticide effectiveness and product performance[14]. The information from the Department of Agriculture, Ministry of Agriculture and Co-operative, Thailand showed that there was an increasing trend of imported pesticides from about 110,000 tons totally in 2007 to approximately 172,000 tons totally in 2013. Among these, herbicides were the major pesticides with the highest proportion of import followed by insecticides, and fungicides, respectively. The most imported herbicides were glyphosate isopropylammonium, paraquat dichloride, 2,4-D sodium salt, 2,4-D dimethyl ammonium, and ametryn. The most imported insecticides were chlorpyrifos, cartap hydrochloride, carbaryl, cypermethin, and carbosulfan. The most imported fungicides were mancozeb, carbendazim, propineb, captan, and copper hydroxide[16-17].

Pesticides work by interfering an essential biological mechanism of the pests. Because all living organisms share many biological mechanisms, their mode of action is not specific to one species. While pesticides kill pests, they may also kill or harm other organisms that are beneficial or at least not undesirable. They may also harm humans who are exposed to pesticides through occupational or home use, eating foods or liquids containing pesticide residue, or inhaling or contacting pesticide-contaminated air[5]. The World Health Organization estimates that there are 3 million cases of pesticide poisoning annually and up to 220,000 deaths, primarily in developing countries. There were about 49,000 to 61,000 reported cases of pesticide intoxication each year with morbidity rate between 76.4 and 96.6 per 100,000 populations during 2007-2013 in Thailand[17]. The application of pesticides is often not very precise, and unintended exposures occur to other organisms in the general area where pesticides are applied. Children, and indeed any young and developing organisms are particularly vulnerable to the harmful effects of pesticides. Even very low levels of exposure during development may have adverse health effects.

Pesticides: the risk to NAFLD

Pesticides are a double-edged sword. They have numerous beneficial effects, crop protection, food and materials preservation, and vector-borne diseases prevention while they are simultaneously toxic by design. The liver is responsible for the detoxification of these xenobiotic compounds primarily through cytochrome P450 enzymes; such as CYP3A and CYP2B families, which initiate the first step of the detoxification process[18- 19]. Therefore, the liver is a target organ for pesticide toxicity. Many pesticides have previously been associated with fatty liver disease and TASH[6, 20].

Herbicides

In Thailand, herbicides considered the largest proportion of imported pesticides, 68,824-137,048 tons annually or 62-79% of imported pesticides during 2007-2013[17]. The treatment of rats with herbicide, glyphosate, alone or in combination with insecticide, dimethoate, or fungicide, zineb, induced oxidative stress in liver, caused loss of mitochondrial transmembrane potential with a concomitant increase of fatty acid peroxidation[21]. The administration of paraquat increased in the lipid peroxide level and changed in lipid composition in the liver of mice[22]. 2,4-dichlorophenoxyacetic (2,4-D), an herbicide in chlorophenoxy group, treated in rats could modify lipidic status, disrupted lipid metabolism and induced hepatic oxidative stress[23]. Moreover, liver steatosis development, increased triglycerides and mitochondrial energetics inhibition, were reported in B6C3F1 mice after long-term simazine, a triazine herbicide, feeding[24]. Long-term exposure to atrazine, a triazine herbicide, contributed the development of steatosis, obesity, insulin resistance, and mitochondrial dysfunction in Sprague-Dawley rats[25]. Chronic exposure of low dose of cypermethrin, pyrethroid insecticide, atrazine and triazine herbicide increased the mRNA levels of key genes that involved in both the *de novo* free fatty acids (FFA) synthesis pathway and the transport of FFA from blood in mice. An increase amount of FFA was partially consumed as energy through β-oxidation in the mitochondria. Some of the FFA were used to synthesize triacylglycerol in the liver by up-regulating primary genes, which resulted in increased triacylglycerol levels and lipid accumulation[26]. The action of triazine herbicides mediated inhibition of the electron transport chain in chloroplasts and thus was as photosynthesis inhibitors. The similarity of plant chloroplasts and mammalian mitochondria may be responsible for the mitochondrial toxicity induced by triazine in animal studies[6].

Insecticides

Imported insecticides in Thailand was ranked second behind herbicides, 16,796-34,672 tons annually or 12-23% of imported pesticides during 2007-2013[17]. Organophosphate insecticides are used in agriculture, homes, gardens and veterinary practices. Several of which are highly toxic, all share a common mechanism of cholinesterase inhibition. Organophosphates are efficiently absorbed by inhalation and ingestion. Chlorpyrifos, an organophosphate insecticide, and carbaryl, a carbamate insecticide, are the top ten imported insecticides in 2013 in Thailand[17]. Subchronic exposure to chlorpyrifos and carbaryl alone, or in combination revealed alternations in a number of metabolites involving the metabolism of glucose, free fatty acids, and amino acids, caused a disturbance in energy and fatty acid metabolism in the liver mitochondria of rats[27]. Methyl parathion and parathion treatment increased lipid peroxidation and oxidative stress in HepG2 cells[28]. Some organophosphates, such as diazinon, fenthion, and methyl parathion, have significant lipid solubility, allowing fat storage with delayed toxicity due to late release[29]. Treatment with individual doses of carbamate insecticides, carbofuran and cartap, caused alterations in the levels of serum lipid parameters. There were marked decreasing in the level of serum high-density lipoprotein where as that of other lipids got elevated. Further, rats exhibited relatively higher impact of pesticides when treated with the compounds in combination[30]. Organochlorine insecticides were intensively used in agriculture to protect cultivated plants. They are high thermodynamic stable and lipid soluble[31-32]. Human exposure occurs primarily via low level food contamination. Hepatic effects, including steatosis, were reported in 32 plant workers poisoned with chlordecone in Virginia. Many of the cases had hepatomegaly, and 12 workers underwent liver biopsy, revealing mild steatosis, portal inflammation, fibrosis, glycogenated nuclei, and lipofuscin accumulation. Interestingly, liver enzymes were repeatedly normal in all subjects, a characteristic commonly observed in TASH[33]. Multiple pesticides including dieldrin, trans-nonachlor (component of chlordane), and heptachlor epoxide (metabolite) were dose dependently associated with increased odds ratios for alanine aminotransferase elevation and suspected NAFLD[6]. The p,p'-dichlorodiphenyldichloroethylene (DDE) as the main metabolite of banned pesticide dichlorodiphenyltrichloroethane (DDT), exposure increased liver levels of palmitic, stearic, oleic, trans fatty, and linoleic acids having altered the n6 and n3 pathways leading to high concentrations of arachidonic acid and DHA (C22:6 n3) in

rats. The results of this study confirm the close relationship between pesticide metabolite and hepatic lipid dysfunction[34]. The hepatotoxic modes of action for organochlorine insecticides are still unknown, but they are related to induce cytochrome P450 enzymes and appear to activate nuclear receptors.

Fungicides

According to US Environmental Protection Agency, global annual fungicide application is nearly 500 million pounds. In Thailand, fungicides were imported about 6,971-12,178 tons annually or 5-11% of imported pesticides in 2007-2013[17]. Mancozeb, a manganese/zinc ethylene-bis-dithiocarbamate fungicide worsened NAFLD in the human HepG2 cell model. The hepatic toxicity of the fungicide exacerbated fatty acid-induced steatosis, as manifested by an increase in intracellular lipid droplet accumulation[35]. Oral administrations with carbendazim induced hepatic lipid metabolism disorder which was characterized by increases of hepatic lipid accumulation and triglyceride levels in mice. Cholesterol, high-density lipoprotein, and low-density lipoprotein levels in serum also increased after carbendazim exposure[36]. Treatment with the organochlorine fungicides captan, dichlofluanid and chlorothalonil induced cytotoxicity and lipid peroxidation in isolated rat hepatocytes[37]. Some azole antifungals including triadimefon, propiconazole and cyproconazole have been previously associated with hepatotoxicity and hepatomegaly in rats[38-39].

Conclusions

Pesticides are designed to kill or harm pests. However, many pesticides can also pose risks to the peoples because they contaminated almost every part of environment. Pesticide residues are found in soil, air, and water. They have been linked to a wide range of human health hazards. TASH is a recently identified form of NAFLD and it is mainly associated with chemical exposure including pesticides. NAFLD is the most common cause of liver disease worldwide and it is strongly associated with insulin resistance and metabolic syndrome. Several potential mechanisms of pesticides, i.e., organophosphate and organochlorine compounds, associated with NAFLD including altered hepatic lipid metabolism, pro-inflammatory cytokine elevation, oxidative stress, and mitochondrial dysfunction. However, NAFLD comprises a wide spectrum of liver damage which results from a ‘multiple-hit’ process. The progressive of NAFLD-associated pesticides from simple steatosis to NASH, advanced fibrosis, and cirrhosis should be further

evaluated, so that more effective diagnostics and therapeutics may be developed.

References

1. Arciello M, Gori M, Maggio R*, et al.* Environmental pollution: a tangible risk for NAFLD pathogenesis. *Int J Mol Sci* 2013; 14: 22052-66.
2. Marchesini G, Bugianesi E, Forlani G*, et al.* Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; 37: 917-23.
3. Chalasani N, Younossi Z, Lavine JE*, et al.* The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; 55: 2005-23.
4. Starley BQ, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. *Hepatology* 2010; 51: 1820-32.
5. Aktar MW, Sengupta D, Chowdhury A. Impact of pesticides use in agriculture: their benefits and hazards. *Interdiscip Toxicol* 2009; 2: 1-12.
6. Wahlang B, Beier JI, Clair HB*, et al.* Toxicant-associated steatohepatitis. *Toxicol Pathol* 2013; 41: 343-60.
7. Puri P, Sanya AJ. Nonalcoholic Fatty Liver Disease: Definitions, Risk Factors, and Workup. *Clin Liver Dis* 2012; 1: 99-103.
8. Day CP, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterol* 1998; 114: 842-5.
9. Dowman JK, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholic fatty liver disease. *QJM* 2010; 103: 71-83.
10. He J, Gao J, Xu M*, et al.* PXR ablation alleviates diet-induced and genetic obesity and insulin resistance in mice. *Diabetes* 2013; 62: 1876-87.
11. Cheng J, Krausz KW, Tanaka N*, et al.* Chronic exposure to rifaximin causes hepatic steatosis in pregnane X receptor-humanized mice. *Toxicol Sci* 2012; 129: 456-68.
12. Angulo P. GI epidemiology: nonalcoholic fatty liver disease. *Aliment Pharmacol Ther* 2007; 25: 883-9.
13. Yilmaz Y. Is nonalcoholic fatty liver disease the hepatic expression of the metabolic syndrome? *World J Hepatol* 2012; 4: 332-4.
14. US Environmental Protection Agency. About Pesticides. Available at https://www.epa.gov/pesticides, accessed Jul 6, 2016.
15. Canadian Centre for Occupational Health and Safety. Pesticides -

General. Available at http://www.ccohs.ca/oshanswers/chemicals/pesticides/general.html, accessed Jul 8, 2016.

16. Panuwet P, Siriwong W, Prapamontol T*, et al.* Agricultural Pesticide Management in Thailand: Situation and Population Health Risk. *Environ Sci Policy* 2012; 17: 72-81.
17. Tawatsin A, Thavara U, Siriyasatien P. Pesticides used in Thailand and toxic effects to human health. *Med Res Arch* 2015: 1-10.
18. Buratti FM, Volpe MT, Meneguz A*, et al.* CYP-specific bioactivation of four organophosphorothioate pesticides by human liver microsomes. *Toxicol Appl Pharmacol* 2003; 186: 143-54.
19. Foxenberg RJ, McGarrigle BP, Knaak JB*, et al.* Human hepatic cytochrome p450-specific metabolism of parathion and chlorpyrifos. *Drug Metab Dispos* 2007; 35: 189-93.
20. Al-Eryani L, Wahlang B, Falkner KC*, et al.* Identification of Environmental Chemicals Associated with the Development of Toxicant-associated Fatty Liver Disease in Rodents. *Toxicol Pathol* 2015; 43: 482-97.
21. Astiz M, de Alaniz MJ, Marra CA. Effect of pesticides on cell survival in liver and brain rat tissues. *Ecotoxicol Environ Saf* 2009; 72: 2025-32.
22. Sato N, Fujii K, Yuge O*, et al.* Changes in lipid peroxidation levels and lipid composition in the lungs, livers, kidneys and brains of mice treated with paraquat. *J Appl Toxicol* 1992; 12: 365-8.
23. Tayeb W, Nakbi A, Cheraief I*, et al.* Alteration of lipid status and lipid metabolism, induction of oxidative stress and lipid peroxidation by 2,4-dichlorophenoxyacetic herbicide in rat liver. *Toxicol Mech Method* 2013; 23: 449-58.
24. Vancova O, Ulicna O, Horecky J*, et al.* Liver steatosis and disorders of mitochondrial oxidative phosphorylation after experimental administration of simazine. *Bratisl Lek Listy* 2000; 101: 423-8.
25. Lim S, Ahn SY, Song IC*, et al.* Chronic exposure to the herbicide, atrazine, causes mitochondrial dysfunction and insulin resistance. *PLoS One* 2009; 4: e5186.
26. Jin Y, Lin X, Miao W*, et al.* Chronic exposure of mice to environmental endocrine-disrupting chemicals disturbs their energy metabolism. *Toxicol Lett* 2014; 225: 392-400.
27. Wang HP, Liang YJ, Long DX*, et al.* Metabolic profiles of serum from rats after subchronic exposure to chlorpyrifos and carbaryl. *Chem Res Toxicol* 2009; 22: 1026-33.

28. Edwards FL, Yedjou CG, Tchounwou PB. Involvement of oxidative stress in methyl parathion and parathion-induced toxicity and genotoxicity to human liver carcinoma (HepG(2)) cells. *Environ Toxicol* 2013; 28: 342-8.
29. Roberts DM, Aaron CK. Management of acute organophosphorus pesticide poisoning. *BMJ* 2007; 334: 629-34.
30. Rai DK, Rai PK, Gupta A*, et al.* Cartap and carbofuran induced alterations in serum lipid profile of Wistar rats. *Indian J Clin Biochem : IJCB* 2009; 24: 198-201.
31. Covaci A, de Boer J, Ryan JJ*, et al.* Distribution of organobrominated and organochlorinated contaminants in Belgian human adipose tissue. *Environ Res* 2002; 88: 210-8.
32. Cruz S, Lino C, Silveira MI. Evaluation of organochlorine pesticide residues in human serum from an urban and two rural populations in Portugal. *Sci Total Environ* 2003; 317: 23-35.
33. Guzelian PS, Vranian G, Boylan JJ*, et al.* Liver structure and function in patients poisoned with chlordecone (Kepone). *Gastroenterol* 1980; 78: 206-13.
34. Rodriguez-Alcala LM, Sa C, Pimentel LL*, et al.* Endocrine Disruptor DDE Associated with a High-Fat Diet Enhances the Impairment of Liver Fatty Acid Composition in Rats. *J Agric Food Chem* 2015; 63: 9341-8.
35. Pirozzi AV, Stellavato A, La Gatta A*, et al.* Mancozeb, a fungicide routinely used in agriculture, worsens nonalcoholic fatty liver disease in the human HepG2 cell model. *Toxicol Lett* 2016; 249: 1-4.
36. Jin Y, Zeng Z, Wu Y*, et al.* Oral Exposure of Mice to Carbendazim Induces Hepatic Lipid Metabolism Disorder and Gut Microbiota Dysbiosis. *Toxicol Sci* 2015; 147: 116-26.
37. Suzuki T, Nojiri H, Isono H*, et al.* Oxidative damages in isolated rat hepatocytes treated with the organochlorine fungicides captan, dichlofluanid and chlorothalonil. *Toxicology* 2004; 204: 97-107.
38. Hester SD, Wolf DC, Nesnow S*, et al.* Transcriptional profiles in liver from rats treated with tumorigenic and non-tumorigenic triazole conazole fungicides:Propiconazole, triadimefon, and myclobutanil. *Toxicol Pathol* 2006; 34: 879-94.
39. Peffer RC, Moggs JG, Pastoor T*, et al.* Mouse liver effects of cyproconazole, a triazole fungicide: role of the constitutive androstane receptor. *Toxicol Sci* 2007; 99: 315-25.