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Effect of Commando Insecticides on Lipid Profile and Liver Enzymes Function in Male Rat

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Abstract
Pesticides are substances used to eliminate or control unwanted pests including weeds, rodents, fungus, germs, and insects. Pesticides were not seen as an issue until recently. Contrarily, the use of these substances was seen as a sign of advancement and modernity and as playing a crucial part in managing agriculture. With this mindset, farmers and the agricultural establishment were seen using more pesticides. Chronic imidacloprid exposure causes oxidative stress and inflammation by altering antioxidant systems and inducing pro-inflammatory cytokine production in the liver and central nervous system of non-target organisms. Imidacloprid is a neonicotinoid insecticide being used extensively for crop protection and pet flea control programmers.

Keywords: Commando Insecticides, Lipid Profile, Liver Enzymes, Male Rat

Introduction
Pesticides are agents used to kill or control undesired pests, such as insects, weeds, rodents, fungi, bacteria or other organisms. Until recently, pesticides were not considered a problem. On the contrary, the use of these compounds was considered to have a vital role in controlling agricultural and a sign of progress and modernization. With this attitude an increase use of pesticides among the agricultural establishment, farmers' was observed. Unfortunately, this increase has not been accompanied by a full understanding of the potential risk and possible adverse health effects to humans, domesticated animals and the environment (Weiss *et al*., 2004) [24]. Since several studies have shown that exposure to pesticides may are mutagenic and induce genotoxic effects (Dulot *et al*., 1985; GarajVrhovac & Zeljezic, 2001; GarajVrhovac & Zeljezic, 2002) [5, 10, 9]
It is fastest growing in sales as insecticide globally because of its low selectivity for insects and apparent safety for humans (Matsuda *et al*., 2001 [15]; Tomizawa & Casida, 2005). Its selective toxicity results from its high affinity to insects nicotinic acetylcholine receptors compared to mammals (Chao & Casida, 1997; Zhang *et al*., 2000; Tomizawa & Casida, 2003) [3, 26, 22].
Imidacloprid, 1[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine, a chloronicotyl is an extensively used insecticide for crop protection in the world wide from the last decade due to its low soil persistence and high insecticidal activity at low application rate (Chao &Casida, 1997) [3].
Imidacloprid is pesticides that are widely used in agriculture, either separately, or in combination (Demsia *et al*., 2007) [4].
Imidacloprid is the most important example of the neonicotinoid insecticides known to target the nicotinic acetylcholine receptor (nAChR) in insects, and potentially in mammals (Duzguner *et al*., 2012) [6].
Imidacloprid is a neonicotinoid insecticide being used extensively for crop protection and pet flea control programmes (Toor *et al*., 2013) [23].

Literature Reviews
Imidacloprid and metalaxyl are two pesticides that are widely used in agriculture, either separately, or in combination. These agents were studied for their possible genotoxic effects with respect to the following cytogenetic end-points: (1) *in vitro* micronucleus (MN) formation and sister-chromatid exchange(SCE) induction in human lymphocytes and (2) *in vivo* micronucleus induction in polychromatic erythrocytes (PCEs) of the rat bone-marrow. The results of the MN analysis indicate that MN frequencies after treatment with both pesticides, separately or as a mixture, do not significantly differ from those in the controls except after treatment with metalaxyl alone at 50 µg/ml ($p < 0.05$). The results of the SCE analysis show that SCE frequencies after treatment with imidacloprid do not differ significantly from those in the controls. A statistically significant increase ($p < 0.05$) in SCE frequency resulted from treatments with metalaxyl at 5, 10 and 100 µg/ml and with the combination of imidacloprid and metalaxyl at 100 and 200 µg/ml. Finally, the *in vivo* micronucleus assay with rat bone-

marrow polychromatic erythrocytes showed a statistically significant effect upon separate treatments with imidacloprid and metalaxyl at doses of 300 mg/kg body weight (b.w.) ($p < 0.01$) or upon combined treatment with 200 mg/Kg b.w. ($p < 0.001$) and 400 mg/kg b.w. ($p < 0.05$) (Demsia *et al*., 2007) [4].

A 90 days oral toxicity study of imidaclopridwas conducted in female rats with doses of 0, 5, 10, 20mg/kg/day. Decrease in the body weight gain was observed at 20mg/kg/day and at necropsy the relative body weights of liver, kidney and adrenal was also significantly increased at this dose level. No mortality occurred during treatment period while food intake was reduced at high dose level. In clinical chemistry parameters high dose of imidacloprid has caused significant elevation of serum AST, ALT, glucose and BUN and decreased the activity of AChE in serum and brain. The spontaneous locomotor activity was also decreased at highest dose exposure where as there were no significant changes in hematological and urine parameters. The brain, liver and kidney of rats exposed with high dose of imidacloprid had showed mild pathological changes. Based on the morphological, biochemical, hematological and neuropathological studies it is evident that imidacloprid has not produced any significant effects at 5 and 10mg/kg/day doses but induced toxicological effects at 20mg/kg/day to female rats. Hence, 10mg/kg/day dose may be considered as no observed effect level (NOEL) for female rats (Bhardwaj *et al*., 2010) [2].

Technical imidaclopridwas evaluated for its effect on ovarian morphology, hormones and antioxidant enzymes in female rats after 90 days oral exposure. Luteinizing hormone (LH), follicle stimulating hormone (FSH) and progesterone levels were estimated in serum of rats and activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and level of reduced glutathione (GSH) and lipid peroxidation (LPO) were estimated in ovary after oral administration of imidacloprid (5, 10, and 20 mg/kg/day) for 90 days. Decreased ovarian weight together with significant patho-morphological changes in follicles, antral follicles and atretic follicles were observed at 20 mg/kg/day. Imidacloprid at 5 and 10 mg/kg/day has not produced any significant changes in ovarian morphology, hormones and antioxidant status of ovary. However, 20 mg/kg/day dose has produced significant alterations in the levels of LH, FSH and progesterone. Similarly significant changes in SOD, CAT, GPx, GSH, and LPO were observed at 20 mg/kg/day dose level. Therefore, it is concluded that imidacloprid at 20 mg/kg/day dose level has produced significant toxicological impact on ovary of female rats as evident by pathomorphological changes, hormonal imbalance and generating oxidative stress and can be considered primarily as Lowest Observed Effect Level (LOEL) for chronic study (Kapoor *et al*., 2011) [13].

In study of Duzguner *et al*. (2012) [6], oxidant and inflammatory responses to chronic exposure of imidacloprid was studied in rats. Wistar rats were randomly allocated into two groups as control and imidacloprid-exposed group ($n = 10$ rat/each group). 1 mg/kg/BW/day imidaclopridwas administrated orally by gavage for 30 days. After exposure, rats were euthanized and liver and brain samples were surgically removed for analyses. Imidacloprid application caused a significant increase in nitric oxide production in brain ($p < 0.05$) and liver ($p < 0.001$). The quantitative analyses of mRNA confirmed the finding that imidacloprid induced the mRNA transcriptions of the three isoforms of nitric oxide synthases (iNOS, eNOS, nNOS) in brain and two isoforms (iNOS, eNOS) in the liver. Exposure to imidacloprid caused significant lipid peroxidation in plasma, brain ($p < 0.001$) and liver ($p < 0.003$). While the superoxide-generating enzyme xanthine oxidase activity was elevated in both tissues ($p < 0.001$), myeloperoxidase activity was increased only in the liver ($p < 0.001$). Antioxidant enzyme activities showed various alterations following exposure, but a significantly depleted antioxidant glutathione level was detected in brain ($p < 0.008$). Evidence of chronic inflammation by imidaclopridwas observed as induction of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-12 and IFN-γ in the liver and brain. In conclusion, chronic imidacloprid exposure causes oxidative stress and inflammation by altering antioxidant systems and inducing pro-inflammatory cytokine production in the liver and central nervous system of non-target organisms.

Imidacloprid is a neonicotinoid insecticide being used extensively for crop protection and pet flea control programmes. The effect of repeated oral administration of two doses of imidcloprid (1/10th and 1/50th of LD_{50} of imidacloprid) on liver of female albino rat was assessed. Histological examination of liver revealed that imidacloprid (1/10th of LD_{50}) treatment resulted in dilations of central vein and sinusoids between hepatocytes howeverimidacloprid (1/50th of LD_{50}) treatment did not induce histopathological changes in liver. Non significant decrease in alkaline phosphatase (AKP) activity was observed in imidacloprid treated rats. Liver aspartate aminotransferase level showed significant increase in higher dose of imidacloprid. Additionally, significant increases in plasma levels of aspatate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (AKP) were observed in the treated rats. The results suggest that the higher doses of imidacloprid at 1/10th of LD_{50} is hepatotoxic as compared to lower dose of 1/50th of LD_{50} of imidacloprid (Toor *et al*., 2013) [23].

Materials and Method

The current study was conducted in the Animal House of the Department of Life Sciences at the Faculty of Science, University of Kufa for the period from 15-9-2018 until 15-1-2019 on a group of male white rats divided into three groups of each group included five animals.

Expermental Animals

Healthy male white albino rat weighting between 210-250g were obtained from the animal house of biology department faculty of science university of Kufa. The animals were housed in clean metabolic cages placed in well ventilated house conditions (Temperature 28-31c, photoperiod, 12 natural light and 12 h dark; humidity; 50-52%). Thetwere also allowed free access to rat pellets and tap water. The cages were cleaned of waste twicw daily at 12 h intervals.

Animals Grouping

A total fifteen male rats were used and were randomly grouped into three group

Group 1 (G1): This group included five rats with a dose of physiological solution for 30 days and was considered as a

control group.
Group 2(G2): This group consisted of five rats that were orally administrated with commando (Imidacloprid 20%) (10 mg / kg / day) with the gavage for 30 days.
Group 3 (G3): This group consisted of five rats that were orally administrated with commando (Imidacloprid 20%) (20 mg / kg / day) with the gavage for 30 days. After end the duration of the dosage was withdrawn blood and separation of the serum for conducted the tests of liver and kidney toxicity.

Conduct Laboratory Tests
Some laboratory tests were carried out on the blood samples that were withdrawn from the groups of rats mentioned above after the end of the period of the dosage of one month after the separation of the serum using the centrifuge and carried out the following biochemical tests.

1. Lipid Profile

a) Total Cholesterol
Cholesterol was determined by chloro-enzymaticmethod for scientist Richmond (1973) and using kits supplied by France Biomerieux Company. This method is based on the degradation of cholesterrol ester by the enzyme cholesterol esterase to fatty acids and the cholesterol, which is oxidized by cholesterol oxidase in present of oxygen to the formation of a compound quinonemine with the help of an enzyme peroxidase and the compound is a red color measured color intensity using the optical spectroscopy device along the wavelength 500 (nm) nanometers and depending on the intensity Absorption of the standard solution The concentration of total cholesterol is calculated in the sample.

b) Measuring the Concentration of Serum Triglycerides
The concentration of triglycerides in the serum was measured using a number of tests prepared by the English Randox company and according to the method adopted by (Allain *et al*., 1974) [1]. The measurement principle is based on the hydrolysis of the triglycerides by libase to calcerol and fatty acids. The resulting cholesterol is oxidized with an enzyme Glycerolkinaseuntil a compound quinonemineis formed. A red color whose color intensity is measured using the spectrophotometer at a wavelength of 520 nm. Depending on the absorption intensity of the standard solution.

c) Measuring the Concentration of High-Density Lipoprotein (HDL-C)
The concentration of HDL cholesterol was measured by following the steps of the instructions accompanying the test prepared by SYRBIOcompany. The principle of measurement is based on the deposition of low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) as a result of the addition of Phosphotungestic acid in present of magnesium ions. After centrifugation, only cholesterol associated with high density lipoproteins. Then take 100 microliters of the starter by following the same steps when measuring total cholesterol concentration.

d) Measuring the Concentration of low-Density Lipoprotein Cholesterol (LDL-C)
Concentration of low-density lipoprotein cholesterol (LDL-C) was measured in blood serum by using the following equation and follow-up by Simon (2006) [20].
LDL-C= TC-(HDL-C+VLDL-C)
VLDL-C= Triglyceride/5.

2. Measurement of Liver Function Enzymes
The use of the kit was provided by a Biomerieux – France Company to determine the value of liver enzymes AST and ALT using a method of Reflotron & Precinorm (2010) [17]. The samples were measured along a 505 nm wavelength and by the unit / ml concentration calculated from application of the sample absorption of the standard samples.

3. Measurement of Kidney Function Enzymes
The function kidney was measured of urea and creatine was measured using the color methodBerthelot (Fawcett). The urease enzyme was used to convert urea by hydrolysis to carbonic acid and ammonia using a method of Reflotron&Precinorm (2011) [16]. The color intensity obtained was then measured at 587 nm and urea was calculated by standard calibration. While creatine was measured using the color Bonsnesmethod based on the principle of creatine reaction with the picric acid under alkaline conditions using a method and was measured on the absorption of 505 nm before and after acidification and this is appropriate with the concentration of creatine.

Statistical Analysis
The mean and standard errors were used in statistical analysis, using the mono-variance test of program SPSS 23 methods and using the complete random design.

Results and Discussion
It is clear from the Fig (1) & (2) that the different doses of the commando pesticide (10 & 20 mg/kg/day) on the other caused a significant decrease) $p<0.05$) in the level of cholesterol and triglycerides in the treatment groups G2 and G3 when compared to the control group.
This effect of the pesticide in lowering the level of cholesterol and triglyceride due to the effect of the pesticide on liver functions where studies have shown that pesticides Insecticides, including commando, cause lipid oxidation and free-radical generation, which play a major role in the damage caused by these pesticides and cause liver dysfunction(Rodríguez-Moro *et al*., 2019) [19]. The reason for the reduction in cholesterol may be due to the effect of the pesticide in the liver causing enzyme changes and this inhibits the enzyme (BuchEButyryl Cholinesterase), which plays an important role in the process of lipid metabolism and cholesterol synthesis. This is agreement with (Kumar Mauryaet *al*., 2019) [14] and also with other studies conducted to evaluate the effect of a number of pesticides on biochemical changes, a decrease in cholesterol and triglycerides (Suarez-Lopez *et al*., 2019) [21]. This effect also has a decrease in LDL (Fig 4) between the treated and control groups (Yang & Park, 2018) [25].
From result in Fig (3) showed that concentrations of commando insecticide not caused any significant effect on

HDL compared with control group.

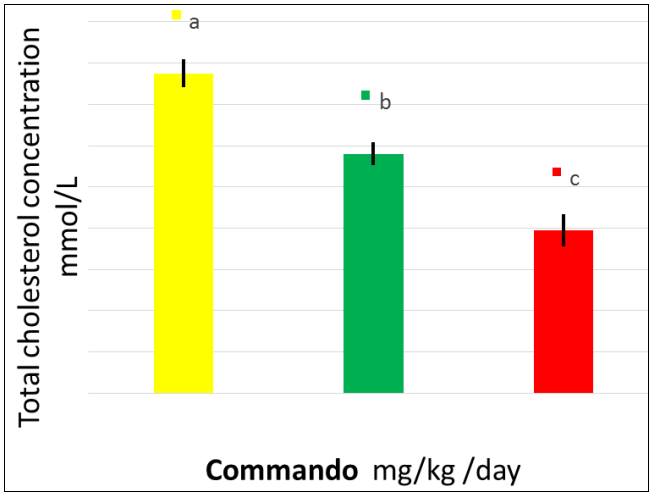

Fig 1: Effect of commando pesticide concentration 10 and 20 mg/kg/day on total cholesterol concentration mmol/L in male rat. Different litter refers to significant differences (P<0.05) according to LSD

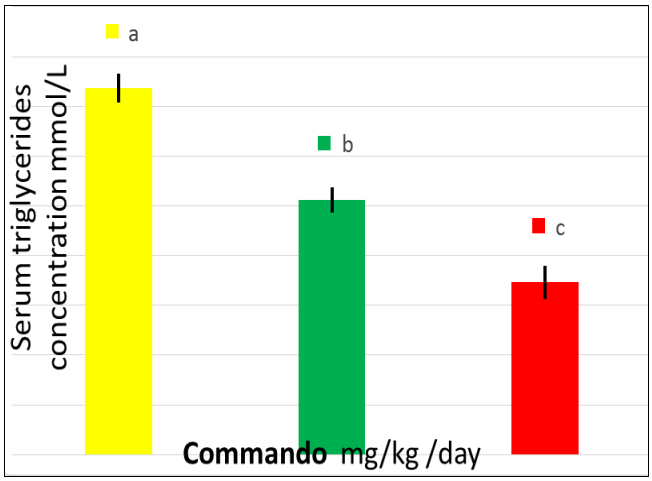

Fig 2: Effect of commando pesticide concentration 10 and 20 mg/kg/day on serum triglycerides concentrationmmol/L in male rat. Different litter refers to significant differences (P<0.05) according to LSD

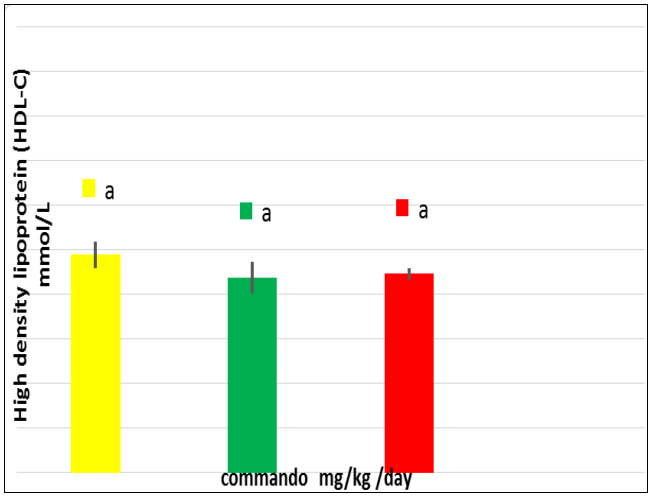

Fig 3: Effect of commando pesticide concentration 10 and 20 mg/kg/day on serum High density lipoprotein (HDL-C) mmol/L in male rat. Different litter refers to significant differences (P<0.05) according to LSD

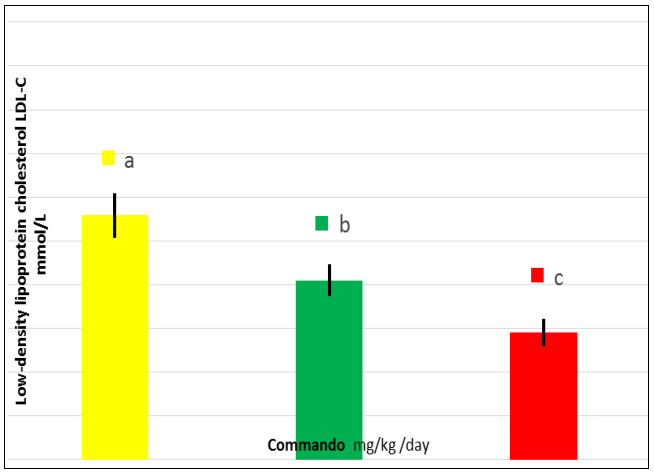

Fig 4: Effect of commando pesticide concentration 10 and 20 mg/kg/day on serum Low density lipoprotein (LDL-C) mmol/L in male rat. Different litter refers to significant differences (P<0.05) according to LSD

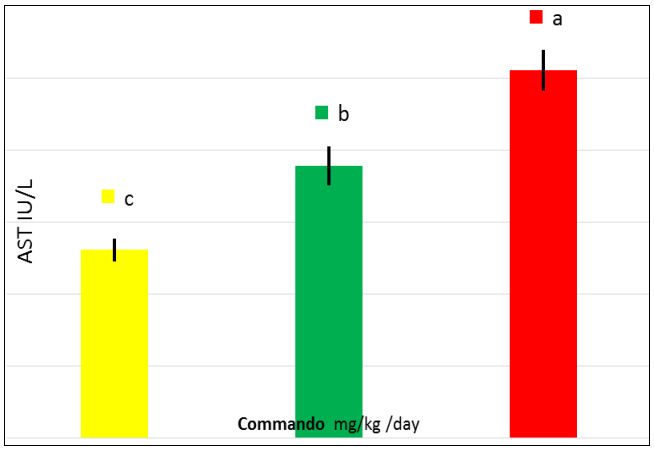

Fig 5: Effect of commando pesticide concentration 10 and 20 mg/kg/day on Aspartate transaminase (AST) in male rat. Different litter refers to significant differences (P<0.05) according to LSD

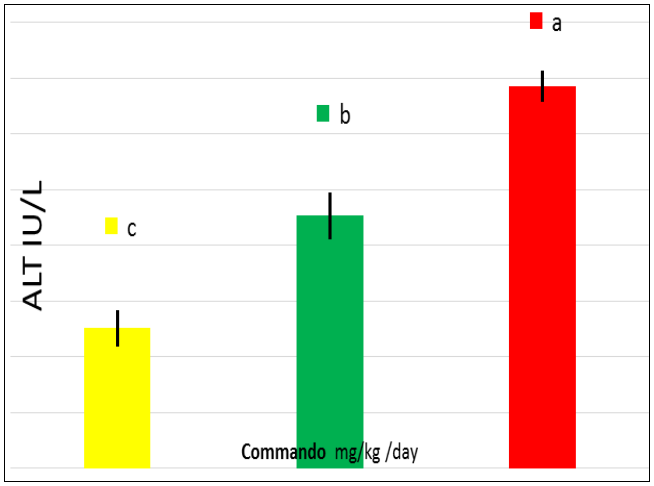

Fig 6: Effect of commando pesticide concentration 10 and 20 mg/kg/day on alanine aminotransferase (ALT) in male rat. Different litter refers to significant differences (P<0.05) according to LSD

The results in Fig (5) and (6) showed that the commando insecticide caused significant increase) p<0.05) in both the liver enzymes (AST & ALT) and the effect of the pesticide

on the liver and the heart, resulting in damage to the tissue as a result of increased permeability of the membranes, which leads these enzymes flow towards the blood stream (Hayat *et al*., 2019) [12]. This result was agreed with both El-Kassabany & Shafika (1995) [8] and Glodny, *et al*. (2009) [11] to increase the level of liver enzymes The cause of the increase in liver enzymes may be due to the effect of the pesticide by increasing the effectiveness of the cells in the cell membranes of the liver cells, causing hepatic cell breakdown and thus releasing their enzymes into the bloodstream (Elhalwagy, *et al*., 2008 [7]; Ahmadian, *et al*., 2018). This explains the increase in hepatic activity.

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