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TOXICOLOGICAL AND BIOCHEMICAL EFFECTS OF IMIDACLOPRID*

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 $\textbf{Keywords: Imidacloprid, pesticide, Pesticide toxicity, Albino\ mice, superoxide}$

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ABSTRACT

Application of pesticides on vegetables will protect them from pest injury, but in another hand will hold pesticide residues in vegetables. These residues have harmful effect against all consumers. In the present study, we took imidacloprid insecticide, because it used widely for combatant vegetable insect control in Iraq. Median lethal dose (LD50) for imidacloprid pesticide was determinant by using Probit analytical method. The evaluation of toxicity carried out by taking 1/10, 1/100, and 1/10000 from the LD50 of imidacloprid insecticide. Orally administration of determinant doses applied on albino mice using gavage-tube for 30 days as sub-chronic exposure. We took both biochemical and hematological parameters, as indicators for insecticide toxicity. The histopathological study been carried out for liver, kidney, and spleen after we sacrifice the mice of each group separately in the end of the sub- chronic orally administration period. ANOVA statistical test carried out for results of imidacloprid biochemical and hematological results. The results varied based on kind of parameters and concentrations, low concentrations dose 1/10, 1/100 and 1/10000 from LD50 has the same or even more adversely effect towards targeted organs, the reason for that attitude due to bio- accumulation effect and the inability of low dose for stimulate the defense systems of body like superoxide dismutase(SOD), whereas, intermediate and high dose concentration able to stimulate body defense systems like SOD.

Abbreviation

SOD, superoxide dismutase; RBC, red blood corpuscle; WBC, white blood corpuscle; MDA, Malondialdehyde; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, Triglyceride

INTRODUCTION

Agriculture in the world has altered greatly in the past one hundred years. Many farmers follow high yield by using low cost energy, plentiful water supply, efficient chemical fertilizers and pesticides (14). Pesticides play as great value in the high productivity accomplished in agriculture through the control of plant or animal pests. Although pesticides have advantages, some have defect, such as potential toxicity to human and other desired species. Exposure of

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general population to pesticide most generally occurs through consuming treated food sources(13).

Despite, good diet contain high percentage of vegetables and fruits show primary factor for reducing the risk of gastrointestinal and breast cancer disease, pesticide residues on vegetables forming possible danger to consumers and have adverse effect on human health (12).

Pesticide is defined as any substances or mixture of substances planned (designed) for avoiding, destroying, repelling, or mitigating any pests (3).

Pesticide residues defined as any substance or mixture of material in food for man or forage for animals resulting from the use of pesticide including any determinant derivatives, such as degradation and conversion products, metabolites, reaction products and uncleanness considered to have significant toxic effect (16).

MATERIALS AND METHODS

Imidacloprid insecticide

Imidacloprid insecticide used in the experiment was commercial insecticide. Its trade name is confidor. The imidacloprid concentration was 200 g/L. The manufacture company is VAPCO Manufacturing CO. Ltd. The vendor of pesticide was the representative of VAPCO Company in Al-Yusufiyah wholesaler market. Imidacloprid chemical name is N-{1-[(6-Chloro-3-pyridyl) methyl]-4, 5-dihydroimidazol-2-yl} nitramide. The chemical formula for pesticide is C9H10ClN5O2 (12).

Calculation LD50 for Imidacloprid Pesticide by using Probit analytical method

The calculation of LD50 for imidacloprid pesticides had been carried out, by using Probit analysis method. According to Randhawa, (21), we should determine LD0 and LD100 for targeted pesticide. Due to the fact that we do not have pure active ingredients for insecticide as we mentioned before, so we need to change the volumetric size of our conventional pesticides that are available in locally markets to get different concentrations of our dissolved active ingredients for carrying out LD0 and LD100 experiment. According to Hendawi, (12), the milting temperature for imidacloprid pesticide is 143.9 °C; this will give us idea that, imidacloprid change from solid phase in to liquid phase in high temperature without deformation its structure. After storage temperature of 54 ± 2 °C for imidacloprid pesticide for 14 days, imidacloprid stay active at 190 g/L from the total active ingredients before the storage process period (11). The concentration of imidacloprid pesticides that fixed on the container is 200g/L, so to calculate the concentration as percent expression, we should apply this equation:

% = (weight of solute (g) / volume of solution (ml)) * 100 % =
$$(190g / 1000 \text{ ml}) * 100$$

So the concentration will become 20%. The container of imidacloprid pesticide considered as stock solution, this is clear because the concentration that is fixed is 200g/L, whereas the container volume was 250ml. By using water bath with safe temperature for example 40 °C, we can get rid of some volume of solvent (methanol), by this technique, we can increase the

concentration of active ingredients and get different concentrations for the determination of LD0 and LD100. We can also dilute the concentration of active ingredients by using proper solvent for example (sterilized water), to decrease the concentration of active ingredients for the same purpose. In both cases, the new concentration calculated by using the following equation:

concentration * volume (before dilution) = concentration * volume (after dilution)

According to World Health Organization, 2020 (23), LD50 for imidacloprid is 450 mg/kg. We took this number as starting point to calculate LD0 and LD100 for targeted pesticide to know the sensitivity of our laboratory animal test to imidacloprid pesticide.

According to World Health Organization, 2020(23), LD50 for imidacloprid is 450 mg/kg.

So:

LD50 = 450 mg/kg

Weight of experimental mice = 25g kg = 1000g

450 mg / x = 1000 g / 25 g x = 11.25 mg

So the LD50 will be 11.25 mg/25g (weight of experimental mice) concentration of our pesticide is 200g/L.

Whereas: g = 1000 mg, L = 1000 ml.

We can express the concentration as: 200000 mg / 1000 ml. The volumetric amount that is equal to 11.25mg is 56.25µl. So we took group consist of ten mice and we gave each one of them 56.25µl by using oral gavage tube as orally intake kind of exposure. The surveillance continued for 24 hours, the results shows no adversely affect appear on the group of mice. Due to this result, we started increasing the concentration.

The experiments shows that LD0 is 4000 mg/kg. Whereas, LD100 is 8000 mg/kg.

According to Randhawa (21), five groups of mice has taken, each consisting of 10 mice. Five different doses has given orally to each individual of the groups. Group 1 gave LD0 concentration, whereas, group five gave LD100 concentration. The remaining three groups has given random concentrations between the LD0 and LD100 values. The animals were monitored for the first 2 hours and then for 6 to record any toxicity symptoms or reaction due to the effect of pesticide exposure, at 24 hours we will calculate the percentage of mortality.

The calculation of corrected percentage had been carried out for 0 and 100 percentage of dead mice as the following formula:

For 0% dead: 100(0.25/n)

For 100% dead: 100(n-0.25/n)

The calculation of doses in (mg/kg) have mentioned in table 1; transformation of percentage mortalities to Probits have mentioned in table 2.

Table1: Converting the imidacloprid doses from (mg/25g) to (mg/kg)

Concentratio n of pesticide mg/L (1)	of pesticide	Orally intake dose(ml) (3)	x(mg/25g) (4)= (1*3)/2)	Kg (5)	Weight 25g (6)	x(mg/kg) (7)=(4*5)/6	Log
200000	1000	0.5	100	1000	25	4000	3.60206
200000	1000	0.6	120	1000	25	4800	3.681241
200000	1000	0.7	140	1000	25	5600	3.748188
200000	1000	0.8	160	1000	25	6400	3.80618
200000	1000	1	200	1000	25	8000	3.90309

Calculation of Slandered Error (SE) of LD50 for imidacloprid pesticide:

The SE of LD50 calculated by using the following formula:

Approx.SE of LD50 = (Log LD84 - Log LD16) / $\sqrt{2}$ N Where N is the number of animal in each group.

Evaluation the Toxicity for Imidacloprid pesticide

According to Shakthi Devan (22), after determination of LD50 for pesticides, the evaluation of toxicity carried out by taking three groups of Albino mice, each group consist of ten mice. The 1st group gave one-tenth of the LD50 and considered as high dose. The 2nd group gave twofold decrease dose from the previous dose and considered intermediate dose. The 3rd group gave twofold decrease dose from the previous dose. We gave the doses for each group by orally intake administration with the help of gavage tube. The following table show the groups with the proper dose for each group:

Table 3: Doses for Evaluation the Toxicity of Imidacloprid:

Group	LD50 mg/kg	Dose mg/kg	Dose mg/25g	Volumetric size of dose
G1	5623.41	1/10=562.34	14.06	70µl
G2	5623.41	1/100=56.23	1.41	7μl
G3	5623.41	1/10000=0.56	0.01	0.05µl(5*10 ⁻²)

From table 3, we can see that the amount of dose for G3 group is very small, so we cannot take it even with micropipette. Therefore, we made series of dilution for maximizing its volume as it explained below.

The tube contain 0.5 µl sterilized water

concentration now 5/100 µl

The orally intake period is one month; the kind of exposure is sub-chronic. Measurement of Total

Cholesterol (TC)

Total cholesterol (TC), triglycerides (TG), High Density Lipoprotein (HDL), and Low Density Lipoprotein (LDL), estimated using the procedure of commercially available kit (Spinreact Spain). Serum cholesterol determined by enzymatic colorimetric method based on the following reaction (1).

Cholesterol in the sample originates a colored complex. The intensity of the color formed is proportional to the cholesterol concentration in the sample. The absorbance (A) of samples and standards against the blank read on 505 nm then the cholesterol in the serum calculated according to the following equation:

Cholesterol (mg/dl) = $[(A) \text{ sample} \div (A) \text{ standard})] \times 200 \text{ (standard conc.)}$

Measurement of Triglycerides Concentration (TG)

Total concentration of triglycerides measured by enzymatic method Fossati, P., and Prencipe, L., 1982(8). Using commercially available kit (Spinreact, Spainy). The intensity of the red colored dye formed, represents triglycerides in the sample. The principal reactions of triglycerides determination are:

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Triglycerides

Glycerol + ATP

glycerol + fatty acids

glycerol-3-

phosphate + ADP Glycerol-3-phosphate +

O<sub>2</sub>dihydroxyacetone phosphate + H<sub>2</sub>O<sub>2</sub>

+ 4-aminoantipyrine

quinoneimine +HCl +H<sub>2</sub>O+4-

chlorophenol
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Samples and standards against the blank absorbance read on 505nm, and then the following equation used to determine the concentration of triglycerides:

Triglycerides (mg/dl) = [A sample \div A standard] \times 200 (standard conc.)

Measurement of High Density Lipoprotein (HDL) Concentration

The chylomicrons, VLDL (very lowdensity lipoproteins) and LDL (low-density lipoproteins) precipitated by addition of Phosphotungestic acid and magnesium chloride. After centrifugation the supernatant fluid contains the HDL fraction, the cholesterol liquicolor test kit.HDL-C was measured by enzymatic method (11). With commercially available kit (Spinreact,Spain). HDL was calculated using the following equation: HDL (mg/dl) = $[(A) \text{ sample} \div (A) \text{ standard}] \times 50$ (calibrator conc.)

Measurement of Serum Low Density Lipoprotein (LDL):

Low-density lipoprotein is determined according to Friedewald 9). Equation: LDL $(mg/dl) = total\ cholesterol - [VLDL + HDL]$

Measurement of Very Low Density Lipoprotein (VLDL)

Very low-density lipoprotein was determined according to the conventional equation (9).

VLDL (mg/dl) = TG/5

Statistical Analysis

for Results

For showing the significant variations between the results of this study, we applied ANOVA table test method for all statistical results of this study because, this is the best method that is used for representing huge amounts of inputs, simple, and has high accuracy over other statistical methods. Lest Significant Differences (LSD) test applied for results in vertical way to know the significant differences between each column values and calculation the probability (P value) to found the percentage of mistake in those results at p ≤ 0.05 .

RESULTS and Discussion

Results of Calculation (LD50) by Using Probit Analysis Method for Imidacloprid Pesticide

Table 4, showed the LD50 for imidacloprid pesticide that has been calculated by using Probit analysis method.

Table 4: Results of Median Lethal Doses of Imidacloprid for the Determination of LD50 after Orally Intake in Albino Mice (n=10)

Group	Dose (mg/kg)	Log dose	Dead %	corrected %	Probits
1(LD ₀)	4000	3.60206	0	2.5	3.69
2	4800	3.681241	40	40	4.75
3	5600	3.748188	50	50	5.00
4	6400	3.80618	90	90	6.28
5 (LD100)	8000	3.90309	100	99.75	7.39

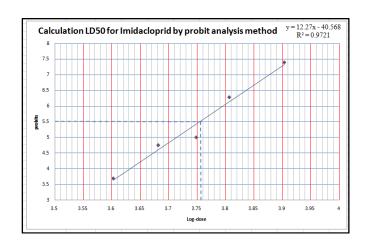


Figure 1: Calculation LD50 for Imidacloprid pesticide by using Probit analysis method.

According to Randhawa, M. A. 2009(21). The Probit values putted

against Log-doses, and then the dose corresponding to 50% of the Probits found out as it appear in figure 1.

From figure 1, the log LD50 = 3.75, so LD50 = 5623.41 mg/kg, the median lethal dose for imidacloprid calculated by Probit analysis method. Calculation of Standard Error (SE) of LD50 for imidacloprid pesticide

According to Randhawa, M. A. 2009(21). The Probits of 84 and 16 from annex 2 are 5.99 and 4.01, approximately 6 and 4. The Log-LD values for the Probits 6 and 4 will be obtain from figure 2. The Log-LD values are 3.79 and 3.63 respectively. So their antilog are LD84 = 6165.95 and LD16 = 4265.80. The formula will be:

SE of LD50 = $(6165.95 - 4265.80) / \sqrt{2*10}$

SE of LD50 = 424.89.

Therefore, LD50 for imidacloprid will be 5623.41 ± 424.89 mg/kg Lipid Profile

Table 5: lipid profile after treatment with imidacloprid pesticide for all groups

Lipid profile Concentration	Ch	Ch TG		LDL	VIDL
control	68.667 b± 2.404	57.000c± 1.528	13.000 bc± 0.577	54.667 b± 2.439	11.400 c ± 0.306
G1	79.667 b± 3.528	83.333 bc± 2.404	15.100 b± 2.108	58.633 b± 2.969	16.667 bc ± 0.481
G2	79.000 b± 1.155	107.000 ab± 11.590	10.833 c± 0.851	55.100 b± 3.086	21.400 ab ± 2.318
G3	142.667 a± 11.348	127.333 a± 14.621	2.567a± 0.921	113.087 a± 8.619	25.467 a ± 2.924
LSD p≤ 0.05	19.859	30.776	4.109	16.189	6.155

Note: Small letters indicate to comparison in column, similar letters are non-Significantly differences between means at ($p \le 0.05$), Using (LSD test)

Table 5 showed the statistical result for the effect of imidacloprid pesticides on lipid profile tests, ANOVA table test results showed significant differences for all kind of treatments.

Cholesterol represent animal sterol that is normally synthesized by the liver (16, 19). Exposed that there is lack of information regarding the alteration of liver under stress of imidacloprid toxicity. They revealed that 96 hours of Bloch fish exposed to imidacloprid pesticide showed significant increase in cholesterol level. Qadir, et al., (20) Showed that the orally administration of imidacloprid for different period time increase the level of cholesterol with comparison with control. Our result regarding cholesterol, compliance with all the scientific evidences that mentioned above. The LSD results showed, significant difference between G3 and control, but there is no significant differences between G1, G2 and control in respect to G3. Due to the previous chapter, G3 represent the low dose in comparison with G1 and G2 that represent high dose and intermediate dose. According to Qadir (20), Carp fish has great ability for neutralized the effect of imidacloprid over long period due to the induction of liver tissue recovery. Our results showed that albino mice have the same ability for the intermediate and high concentration doses, G1 and G2 groups, but not low concentration dose. That happened because low

concentration dose, unable for induction liver tissue recovery. Therefore, the effect of bioaccumulation will be great.

Triglyceride (TG) occurring naturally in animal and vegetables tissue, it is considered an important source of energy forming of fat, it is represent part from blood serum (21). Qadir et al., (20) Showed that the effect of exposure Labeo rohita fish to imidacloprid for 2-4 days showed significant increase in the levels of triglyceride (18). Explained that generally, the exposed of farmers to pesticides increase the level of triglyceride. Our statistical results showed significant increase with TG level in comparison with control, so the results compliance with the scientific facts that mentioned above in this category. LSD test regarding TG exposed that G3 and G2 have significant effect in comparison with control, whereas, G1 has no significant effect with control in respect to G2 and G3. According to García-García, et al., (10). They mentioned that oxidative stress caused by pesticides may induce weakness in the metabolism of lipid, this will cause increase the level of TG. Annabi et al., (2), Mentioned that long time of rats exposure to imidacloprid create kind of adaptation effect to stress of imidacloprid, this effect happed due to the induction of hypothalamic-pituitary-adrenal that will play important role in reduce the level of TG that is increased due to the effect of imidacloprid, This result compliance with the G1 result regarding TG with respect to G2 and G3.

High-density lipoprotein (HDL) is complex particles composed of multiple proteinswhich transport all fat molecules (lipids) around the body within the water outside cells (24). Due to Bal et al., (4), Neonicotinoid pesticide have great effect on increase the level of cholesterol due to their oxidative stress, cholesterol is the main precursor for steroidogenesis that is produce in the liver by HDL and low density lipoprotein(LDL). LDL like HDL in their function. Duzguner and Erdogan, (6), mentioned that imidacloprid has adversely affect against liver due to its oxidative stress against liver cell tissue, so this will affect the level of HDL and LDL, this scientific fact compliance with our results mentioned in table (5) regarding HDL, LDL that show significant effect of imidacloprid on HDL, LDL in comparison with control. LSD result that is mentioned in table (5) regarding HDL, LDL, both show significant effect for G3 in comparison with control with respect to G1 and G2, but G1 and G2 showed no significant effect with control in respect to G3, according to EL-Gendy et al., (7), Vitamin C has great impact on ameliorate oxidative damage of imidacloprid against liver tissue. Our mice took pellet as forage, one of the most important components for pellet is silage, silage composed of fermented plant residues, one of the residues that are used in silage is citrus residues, citrus residues rich in vitamin C. Therefore, this is the scientific explanation of LSD results regarding HDL, LDL.

Very low-density lipoprotein (VLDL) is one of the five major groups of lipoproteins that enable fats and cholesterol to move within the water-based solution of the blood stream, it is type of lipoproteins made by liver Dashti et al. (5). Mondal et al., (17) mentioned that liver is the major organ that affect with neonicotinoid pesticides due to its ability for detoxifying toxic substances. Our results in table 5 regarding VLDL compliance with this scientific fact and that will explain the increase in the level of VLDL in comparison with control. Mondal et al., (17) Also mentioned that there is no

significant differences between some orally administration of neonicotinoid pesticide in comparison with control. Our results in table (5) regarding VLDL compliance with this scientific fact, because there is significance differences between G2 and G3 with control in respect to G1, but there no significant effect in G1 with control in respect to G2 and G3 that happened due to the adaptation effect phenomena that explained before.

Figure 2 compare between all lipid profile parameters that affected by orally administration of imidacloprid, it also shows the severity of effect.

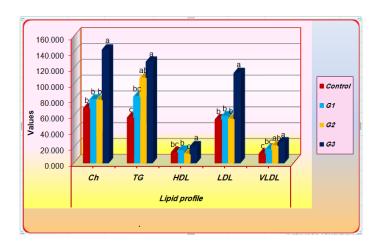


Figure 2: show the effect of imidacloprid on lipid profile. **REFERENCES**

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التأثيرات السمية والكيميائية الحياتية في مبيد الاميداكلوبرد* معدي1

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