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Characterization and in-vivo toxicological evaluation of imidacloprid nanoemulsion in rats

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ABSTRACT

Acute pesticide poisoning is an important public health problem worldwide and accounts for a significant number of deaths occurring each year. The present article aimed to investigate toxic effects of imidacloprid (IMD) nanoemulsion formulated using ultrasound dispersion technique and characterized using FTIR, TEM anddynamic light scattering in adult rats. The synthesized Nano-emulsion droplets are mainly spherical and their sizes ranged between (19nm –128 nm) with zeta potential of −38.8± 0.mV. Also, The median lethal dose (LD_{50}) of nano imidacloprid in rats was 39 mg/kg body weight. Administration of different doses of 3, 1.4, and 0.8 mg.kg mg/kg b.wt. of IMD Nano emulsion to rats for 21 days, adversely affects the body weight and weight gain, and resulted in a significant increase in serum ALT, AST activities, glucose, Creatinine, urea and cholesterol concentrations, as well as reduced serum total proteins, Albumin and globulin as compared to control rats. The results suggest that treatment with IMD nanoemulsion adversely affects the liver & kidney functions which are confirmed by the histopathological findings. Nanoemulsion forms and also increases the DNA damage as confirmed by the comet test.

Keywords: Imidacloprid, nanoemulsion, toxicity, comet assay, rats.

INTRODUCTION: Rapid progress occurred nowadays in the field of nanotechnology in different and multiple fields as environmental protection, medical therapy, and hygiene besides designing a variety of biotechnological applications. One of the main aims for nanotechnology application in agriculture (Pesticides and Insecticides nano-conversion) is to reduce the pest's problem and increasing insecticide efficiency. Most pesticide compounds are insoluble in aqueous media, which obstruct the development of environment-friendly formulations and their efficient application. Nanotechnology may become an innovative strategy to produce nano-formulations for increasing the solubility and efficacy of insoluble pesticides. Decreasing the particle size of pesticide could effectively enhance its dissolution performance; however, the obvious change appears only when the particle size is in the nanoscale. Nanoemulsion is a complex system that contains an oil phase with the presence of surfactant and water. Nowadays this form of nanoemulsion becomes a topic for many studies, uses, and applications (Salim *et al.*, 2011). In this study, the selected insecticides to be converted into nanoemulsion form are the imidacloprid as model pesticides. Imidacloprid has a novel mechanism of action on the nervous system through parasympathomimetic effect causing paralysis then death. From all these facts and even the importance and efficacy of Nano synthesis of different insecticides or drugs; there is no sufficient study about the toxicity of nanomaterial's on animal or human body functions. From this point of view in this study, we are trying to explore the possible toxicity of insecticides converted into nano-preparation for safety use of nano-preparation from insecticides. Imidacloprid is a systemic chloronicotinyl insecticide with broad efficacy against different insects. It acts by increasing the levels of acetylcholine in the nervous system, causing paralysis and eventually death (Wan-Jun *et al.*, 2010). Many pest control strategies have been used to reduce and control the leaching of pesticides from their formulation, for instance, Nano-synthesis for more efficacies, decreasing the dose, or even increasing the efficacy (Garrido-Herrera *et al.*, 2006). However to date, the toxicity or safety of imidacloprid in Nanosystems has not been published in-vivo.

OBJECTIVES: On this context, the aim of the present study was to develop an insecticidal nanoemulsion containing Imidacloprid and to determine the effects and safety of imidaclopridnano-prepration (Nanoemulsion) on rats, and establish whether the Nano formulation of imidacloprid was less or more toxic for rats than the common formulation imidacloprid form.

MATERIALS AND METHODS: Nanoemulsion synthesis of imidacloprid: Nanoemulsion was provided by Nano Tech Egypt for Photo-Electronics, City of 6 October, Al Giza, Egypt, and was prepared according to Shahavi *et al.* (2019) using tween 80 and span80 surfactants as an emulsifying agent. Tween, span 80, and Imidacloprid were mixed and sonicated for 30 min using an ultrasonic processor.

Droplet size measurements: The particle size (z-average diameter) and size distribution (PDI) of the nanoemulsion droplet were measured by a Malvern (Malvern Instruments Ltd, at 298 K.), Nano Zeta Sizer through dynamic light scatter (DLS). Before measuring the emulsion was diluted 1000 times with Q-distilled water to prevention the of light scattering and each sample was measured three times for accuracy.

Stability of Nano- emulsion measurement: The stability of the Nano imidacloprid was assessed in terms of Zeta potential using the particle size analyzer (Malvern Instruments Ltd) allowing running from -200mV to +200mV and plotting the data in the

graph. Before measuring, the emulsion was diluted 1000 times with Q- distilled water to prevention light scattering, and each sample was measured three times for accuracy.

Transmission electron microscopy (TEM): Transmission electron microscope (TEM, JEOL-JEM 2100) was used to obtain more detailed information about the morphology of the nanoemulsion droplets.

Fourier-transform infrared spectroscopy: The vibrations of the nanoemulsion chemical bonds were examined by Fourier Transform Infra-red (FT-IR, Bruker Vertex 70); used for determination and evaluation of the bonds present in the imidaclopridnanoemulsion form.

Determination of LD_{50} of the imidacloprid nanoemulsion: The imidacloprid nanoemulsion was orally administrated in different doses to find out the range of doses that cause zero and 100% mortality of animals. LD_{50} was determined by the administration of the imidacloprid nanoemulsion in different doses 10, 20, 40, 50, 60, and 70mg/kg orally, in six groups, 5 animals per group. After administration of the tested imidacloprid nanoemulsion, animals were observed individually every hour 24 hrs. and Behavior and clinical symptoms of animals were noted throughout the experiment. The **LD_{50}** was calculated according to the following formula (equation 1):

$$1.\ \mathrm{LD50} = \mathrm{DM} - \frac{\sum(\mathrm{AxB})}{\mathrm{N}}$$

Where:

DM: The dose caused 100 % mortality or largest doses which kill all animals

A: Constant factor between two successive doses, Dose difference between 2 successive groups

B: Mean number of dead animals between two successive groups. **N:** Number of animals in each group,

∑: Summation of multiplying A and B.

Experimental animals and treatment protocol: Imidacloprid-nanoemulsion doses were selected based on its LD_{50}, which is determined to be 39 mg/kg body weight; three different doses, i.e., high dose, $1/13^{th}$ of LD_{50} (3mg/kg), medium dose, $1/27^{th}$ of LD_{50} (1.4 mg/kg) and low dose $1/50^{th}$ of LD_{50} (0.8mg/kg) were selected. The commercial Imidacloprid 35% liquid form produced by *Cairo Company for chemicals* was purchased from the local market in Cairo, Egypt. A total of 36 adult male albino rats (average body weight 150 to 250gm) have been used. Animals used in this experiment obtained from the physiology department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt. Animals were treated ethically as recommended by a committee of animal rights and care as legislation requires in Beni-Suef University. All animals left one week without treatment before the start of the experiment and watered on free access of water and a standard diet.

Rats were divided into six equal groups (6 rat/group) as follow:

Group 1 served as the control.

Group 2 treated with imidacloprid common preparation 45 mg/kg b.wt.

Group 3 treated with imidacloprid common preparation 90 mg/kg b.wt.

Group 4 treated with imidacloprid nanoemulsion0.8 mg/kg b.wt.

Group 5 treated with imidacloprid nanoemulsion1.4 mg/kg b.wt.

Group 6 treated with imidacloprid nanoemulsion 3 mg/kg b.wt.

These different doses were administered by oral gavage every day for 21 days. At the end of the experiment, rats were sacrificed by cervical dislocation after anesthesia with Ketamine (90mg/kg b.wt.) and Xylazine (5mg/kg b.wt.) combinations with a ratio 1:1, and blood were collected without any anticoagulant. The serum separation was used for studying the serum biochemical analysis.

Biochemical analysis: Blood samples were collected from all rats of the control and experimental group. The blood samples were centrifuged at 1500 × g for 10 min after standing for 2 h. The serum was separated and immediately frozen to −80 ◦C until analysis. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, albumin, glucose, total cholesterol were measured through Konelab20-fully automated biochemical analyzer (Thermo scientific, Japan) using standard diagnostic kits and analytical grade reagents [Biosystems Egyptian Company for biotechnology (S.A.E), Egypt] according to manufacturer's instructions

Estimation of the genotoxicity effect on liver DNA: The comet assay in liver tissue was performed following the technique described by Miyamae *et al.* (1997). The liver was removed from the rats after sacrificing, minced, and suspended at ~100 mg/mL in chilled homogenizing buffer (pH 7.5) and homogenized gently at a speed of 500–800 rpm. For each slide preparation, 10µL of liver homogenate was used. Three slides were prepared for each experimental condition. In brief, microscope slides were pre-coated with 120µL of 0.75% NMA in PBS and allowed to solidify overnight at 37 °C after putting a coverslip for a uniform layer. The second layer was prepared after pipetting 120µL of 0.37% LMA containing blood or liver cells on the pre-coated slides and dried at 4°C for 10 min. The third layer of plain 0.37% LMA (120µL) was applied and a coverslip was quickly put to get an even layer and dried at 4°C. After removing the coverslip, the slides were immersed in chilled lysis buffer (2.5M NaCl, 0.1M Na_2EDTA, 0.2 M NaOH, 1% Triton X-100, 10% DMSO, pH 10.0) for 10h at 4°C. The slides were pre-soaked for 20 min in alkaline buffer (10M NaOH, 200 mM Na_2EDTA, pH > 13.0) and then electrophoresis was performed at 25V adjusted at 300mA for 20 min. The slides were neutralized twice in 0.4M Tris buffer, pH 7.5, for 5 min and once in absolute methanol for 5 min. Coded slides were scored after staining with ethidium bromide (20µg/mL) using a fluorescence microscope (Olympus, Shinjuku-ku, Tokyo, Japan) with a blue (488 nm) excitation filter and yellow (515 nm) emission (barrier) filter at ×400 magnification. A total of 150 randomly selected cells per rat (50 cells per slide) were used to measure the amount of DNA damage and expressed as a percentage of DNA in the comet tail. Quantification of DNA breakage was realized by using a Comet Image Analysis System, version Komet 5.5 (Single cell Gel Electrophoresis analysis company, Andor Technology 2005, Kinetic Imaging Ltd., Nottingham, UKt (Fatma I. Abo El-Ela *et al.*, 2016).

Tail moment = length of DNA migration (µm) X percentage (%) of migrated DNA.

Histopathological examination: For histopathological examination, liver, spleen, kidney, and intestine were fixed in 10% neutral buffered formalin, dehydrated in ascending series of ethanol, cleared in xylene, and embedded paraffin wax. Sections in size of 5µm were prepared, stained with Hematoxylin and Eosin (HE) 12, and examined under a light microscope at 200 and 400× magnifications (Yanai *et al.*, 1998b).

Statistical analysis: Statistical evaluation for all the data was calculated through (One-way ANOVA) and post comparison was carried out with LSD test using SPSS (Statistical Package for Social Sciences) version 17.00. The results were expressed as Mean±SD and the values of *p<0.05* were considered statistically significant (Schneider *et al.*, 2012).

RESULTS: The FT-IR spectra of imidacloprid and imidacloprid nanoemulsion are shown in figure 1a and b, and the selected FT-IR data are summarized in table 1, where it can be seen that the characteristic absorption peaks of imidacloprid at 1562cm^{-1} for pyridine stretch, 1435 cm^{-1} for C=N stretch and 1102cm^{-1} for C—Cl stretch appear in the spectrum of imidacloprid nanoemulsion indicating that imidacloprid molecules were loaded in modified imidacloprid.

Nano-imidacloprid	Parental imidacloprid	assignment
3430	-	N-H merged with OH stretch
2983	2905	C-H stretch
-	3353	N-H stretch
1562, 1435	1562, 1435	Pyridine C=N stretch
1102	1102	Pyridine C-Cl stretch

Table 1: Summary of selected IR data (wavelength/cm^{-1}) of imidacloprid and its nanoemulsion between 4000 – 450cm^{-1}

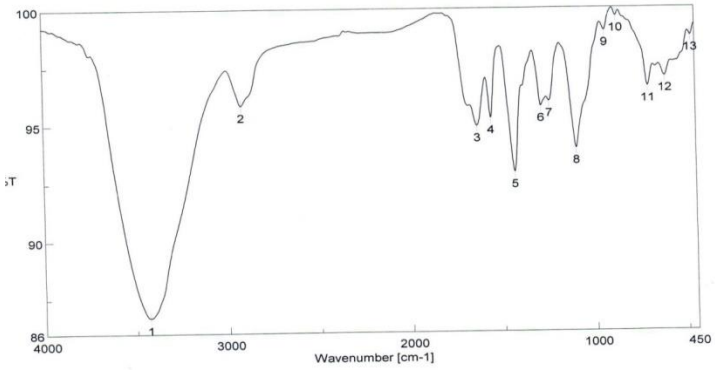

Figure 1a: FTIR spectra of imidacloprid Nanoemulsion with its characteristicpeaks.

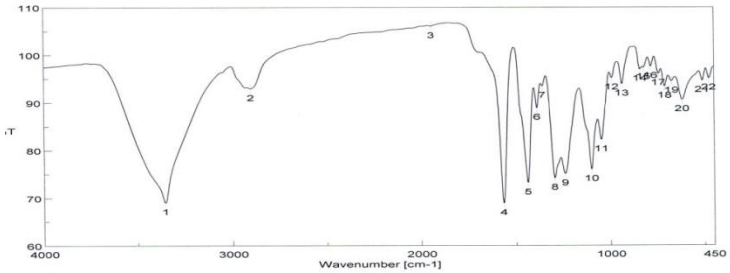

Figure 1b: FTIR spectra of imidacloprid with its characteristic peaks.

The peak appeared at 3353cm^{-1}, associated with the stretching of N–H in parental imidacloprid may be merged with the OH stretching vibrations of tween/ span80 and observed at 3430cm^{-1}. These obvious changes suggest that imidacloprid and the emulsifiers interacted strongly and that imidacloprid became part of the imidacloprid nanoemulsion molecular self-assembly. Transmission Electron Microscopy, as well as Light scattering techniques, were used to evaluate the size, zeta potential, and morphology of imidacloprid nanoemulsion. As shown in figure 3 all the synthesized Nano-emulsion droplets are mainly spherical in shape and their sizes ranged between (19nm –128 nm) with a zeta potential of –38.8± 0.Mv (figure 2), indicating higher physical stability of the prepared Nano-emulsion.

Biochemical toxicological studies: Tremors, rapid respiration, arched back, convulsions, and coma followed by death are the major symptoms of toxicity observed following administration of high doses of imidacloprid nanoemulsion. The LD_{50} was calculated to be 39 mg/kg b.wt. and 100% mortality (LD_{100}) was achieved at a dose of 70 mg/kg b.wt.

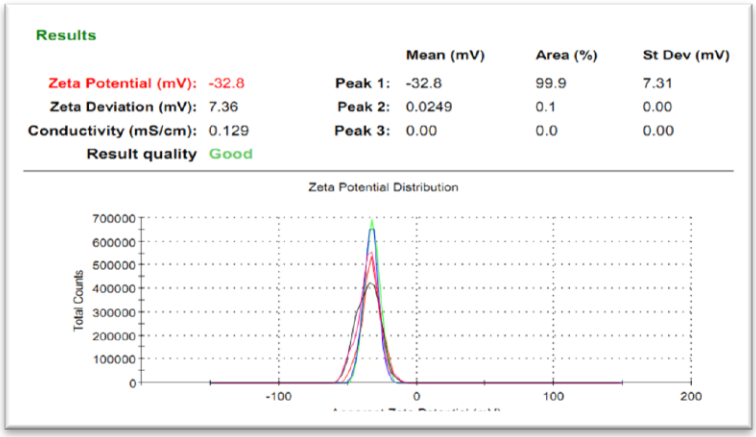

Figure 2: The zeta potential of imidacloprid nanoemulsion at different pH.

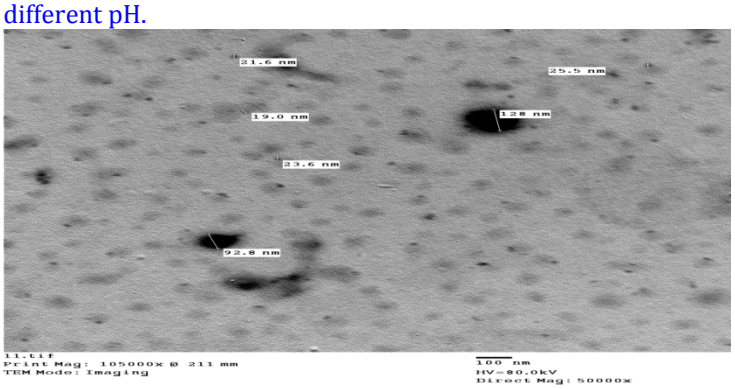

Figure 3: TEM images for imidacloprid nanoemulsion.

The subacute commercial imidacloprid (45mg/kg b.wt. ($p<0.05$), 90mg/kg b.wt of commercial imidacloprid) and imidacloprid nanoemulsion treatments (0.8, and 1.4 mg/kg b.wt.) caused a significant (($p<0.05$) dose-dependent decrease in food intake (table 2), and mean body weight compared to control. Even a sharp ($p<0.05$) decrease in body weight was observed in 3 mg/kg b.wt. nano-imidcloprid treatment compared to other treatment groups (figure 4).

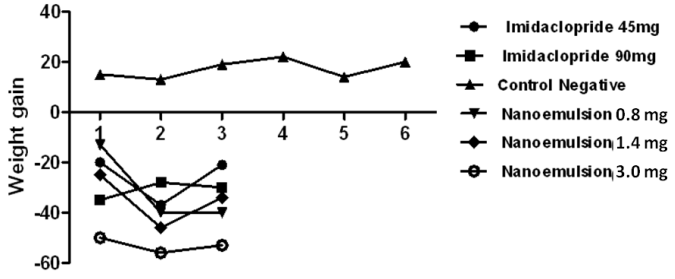

Figure 4: The effects of both imidacloprid commercial form and imidacloprid nanoemulsion on the rats weight gain.

Group	Dose (mg/kg) b.wt	Weekly Food Intake (Week) Mean ± SE		
		1st	2nd	3rd
G4: Nano-Imidacloprid	0.8	0.6±10.33	0.33±8.66	0.33±10.66
G5:Nano-Imidacloprid	1.4	0.57±10.00	1.33±9.66	1.5±9.00
G6: Nano-Imidacloprid	3	0.33±7.66	0.33±5.66	1.1±7.00
G3: Imidacloprid	45	0.8^{d}±22.33	0.57^{d}±23.00	0.57^{d}±23.00
G3: Imidacloprid	90	0.5^{e}±17.00	0.66^{e}±16.33	0.33^{e}±15.33
G1: Control Negative	0.1 ml/100g	0.83^{a}±42.16	1.1^{a}±46.1	1.2^{a}±49.33

Table 2: Effects of imidacloprid nanoemulsion on the food intake in rats.

Many effects on serum clinical chemistry (table 4) were encountered such as a significant ($p<0.05$)dose-dependent increase in serum ALT, AST , activities, glucose, Creatinine, urea, and cholesterol concentrations compared to the control group, and this elevation was being highest in the rats of group 6. On the other hand, Serum total proteins, Albumin, and globulin levels were significantly decreased compared to control animals.

Genotoxicity: Comet assay is a simple, rapid, and sensitive technique for the detection of DNA damage in cells. It is used as a bio-monitoring tool for the assessment of genotoxicity. Effect of different doses of Nano-imidacloprid and commercial preparation on DNA fragmentation in liver cells of the rat using comet assay is shown in figure 5.

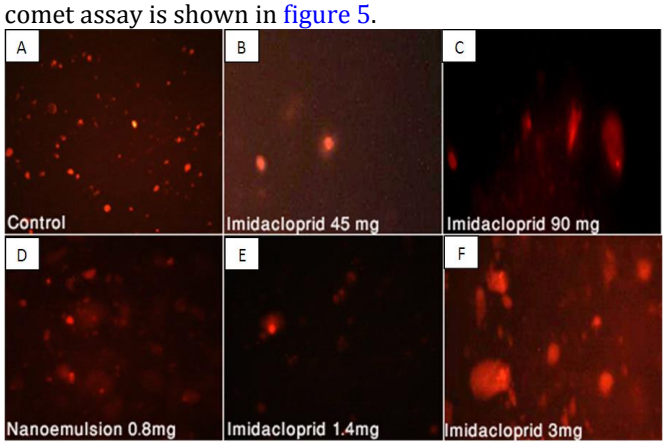

Figure 5: Effects of Imidacloprid in normal and Nano form alone on the DNA of Rat's hepatocytes (tail moment).

In the control group figure A greater number of cells with intact DNA and few comets with very short tail length were seen. The extent of DNA damage along with the number of comets was increased with increased doses of Nano- imidacloprid. In group 4 (0.8mg/kg b.wt) (figure 5d), comets with large tail lengths were observed indicating severe DNA damage. More prominent fragmented DNA (damaged) with larger tail length was found in 3 mg/kg b.wt group of Nano-imidacloprid (figure 5e) and 90 mg/kg b.wt of commercial groups (figure 5c) group compared to control and 45mg/kg b.wt commercial groups, reflecting a great degree of DNA damage in liver cells. The tail moment is one of the most appropriate parameters used to measure DNA damage.as shown in Table 3, the extent of DNA damage measured by tail moment increased rapidly in nano- groups when compared to other groups.

Histopathological results: Histopathological examination of the liver of control rats showed histological structure with normally appeared central vein, hepatocytes and preserved lobular architecture (figure 6).

Group	Dose (mg/kg) b.wt	Tail moment
Imidacloprid	45	0.9^{d}
Imidacloprid	90	1.5^{b}
Imidacloprid/ Nanoemulsion	0.8	0.7^{e}
Imidacloprid/ Nanoemulsion	1.4	1.1^{c}
Imidacloprid/Nanoemulsion	3	1.9^{a}
Control Negative	0.1mL/100g	0.3

Table 3: Effects of Imidacloprid in normal and Nanoemulsion form on the DNA of Rat's hepatocytes (tail moment) in rats. (Mean ± S.E.).

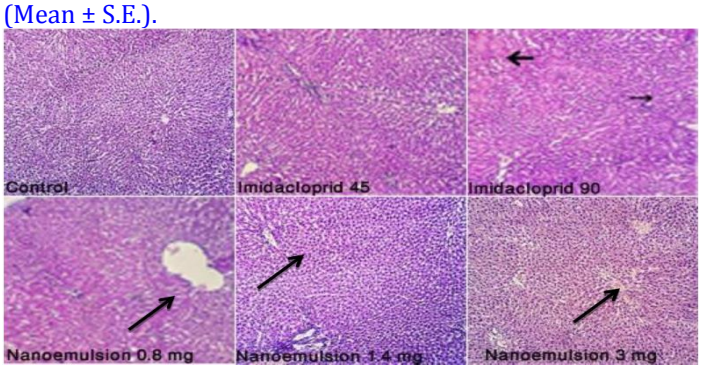

Figure 6: stopathological investigation of Liver Normal histological structure appeared in control non-treated group with normal hepatocyte, lobular architecture and central vein. The IMD nanoemulsion groups showed hepatocytes hydropic degeneration and areas of centri-lobular necrosis (black arrow) which increase with dose. In IMD groups also the histological investigation showed also hepatocytes degenerative changes but in a degree lower than IMD nanoemulsion form. However commercial and Nano-imidacloprid treated rat liver showed hepatocytes hydropic degeneration and areas of centrilobular necrosis which increases in a dose-dependent manner. Histological examination of the Kidney showed regular glomeruli and renal tubules with moderate hydropic degenerative changes in the control group (black arrow). In rat-treated groups with both forms of IMD showed renal tubules with focal necrosis, in addition to marked hydropic degenerative changes (figure 7 and 8) (black arrow).

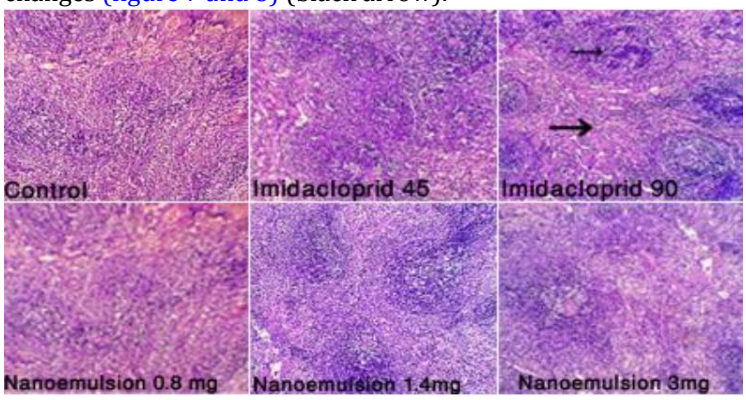

Figure 7: Histological examination of the spleen of Control showed normal white and red pulp with predominantly non-activated follicles and unremarkable red pulp. In IMD nanoemulsion groups showed severe lymphocytic degeneration in the white pulp but moderate in IMD only(black arrow).

In the intestine and Spleen, both showed necrotic and inflammatory cells appeared in the imidacloprid groups of both forms (figure 9 and 10).

Group	Dose (mg/kg) b.wt	ALT (IU/L)	AST (IU/L)	Urea (mg/dl)	Creatinine (mg/dl)	Cholesterol (mg/dl)	Glucose (mg/dl)	Total-protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Imidacloprid	45	252± 3.06^{c}	360± 5.7^{d}	50±5.2^{c}	0.79±0.09^{b}	110±8.50^{d}	150±2.08^{d}	4.8±0.25^{c}	2.3±0.15^{b}	2.5±0.10^{b}	0.90±0.03^{c}
Imidacloprid	90	364± 1.00^{a}	442± 6.6^{b}	61± 1.53^{b}	0.90±0.01^{a}	126±5.51^{a}	176±4.4^{b}	3.7±0.25^{d}	1.7±0.15^{c}	2.0±0.10^{c}	0.86±0.03^{c}
Nano-Imidacloprid	0.8	199± 1.50^{d}	396± 6.04^{c}	43±0.58^{d}	0.83±0.06^{a}	115±0.04^{c}	144±0.03^{e}	5.1±0.35^{b}	2.7±0.20^{b}	2.4±0.15^{b}	1.11±0.02^{b}
Nano-Imidacloprid	1.4	262± 2.50^{b}	397± 7.04^{c}	62±6.08^{b}	0.87±0.06^{a}	120±0.01^{b}	157±0.06^{c}	3.9±0.12^{d}	1.8±0.10^{c}	2.1±0.15^{c}	0.84±0.01^{c}
Nano-Imidacloprid	3	366± 3.10^{a}	472± 5.04^{a}	74±2.52^{a}	0.90±0.06^{a}	128±0.04^{a}	183±0.03^{a}	3.1±0.15^{e}	1.3±0.06^{c}	1.9±0.15^{d}	0.68±0.07^{d}
Control Negative	0.1 ml/100g	39±1.00^{e}	137± 9.6^{e}	26±1.53^{e}	0.57±0.06^{c}	79±2.08^{e}	98±5.51^{f}	7.5±0.64^{a}	4.4±0.59^{a}	3.0±0.06^{a}	1.50±0.01^{a}

Table 4: Effects of imidacloprid in normal and nanoemulsion form on the serum biochemical changes in rats. (Mean ± S.E.)

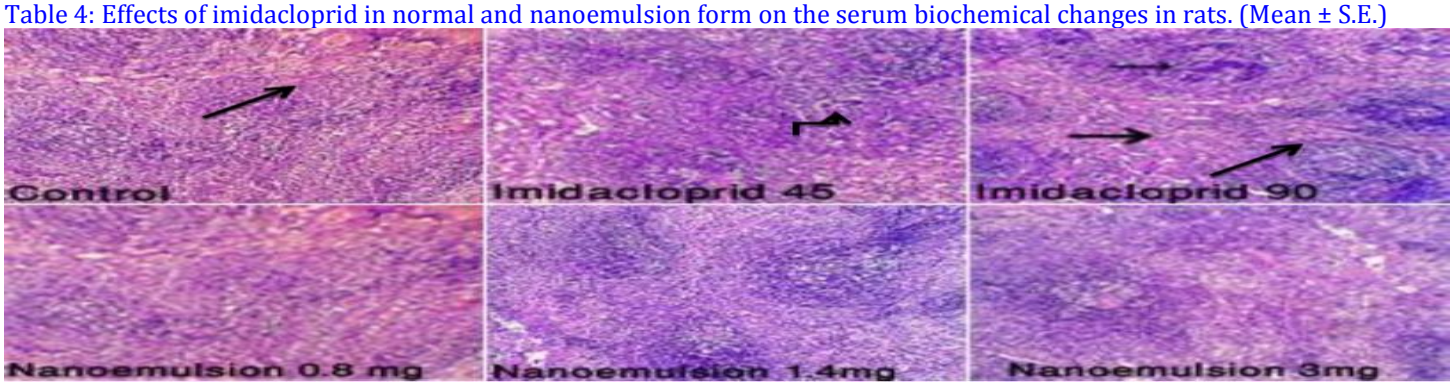

Figure 8: Normal histological structure of the Kidney showed regular glomeruli and renal tubules with moderate hydropic degenerative changes in control group. In IMD Nanoemulsion groups appeared severe focal necrosis increased with dose with marked hydropic degeneration which also appeared in IMD groups but in aa lower degree (Black arrow).

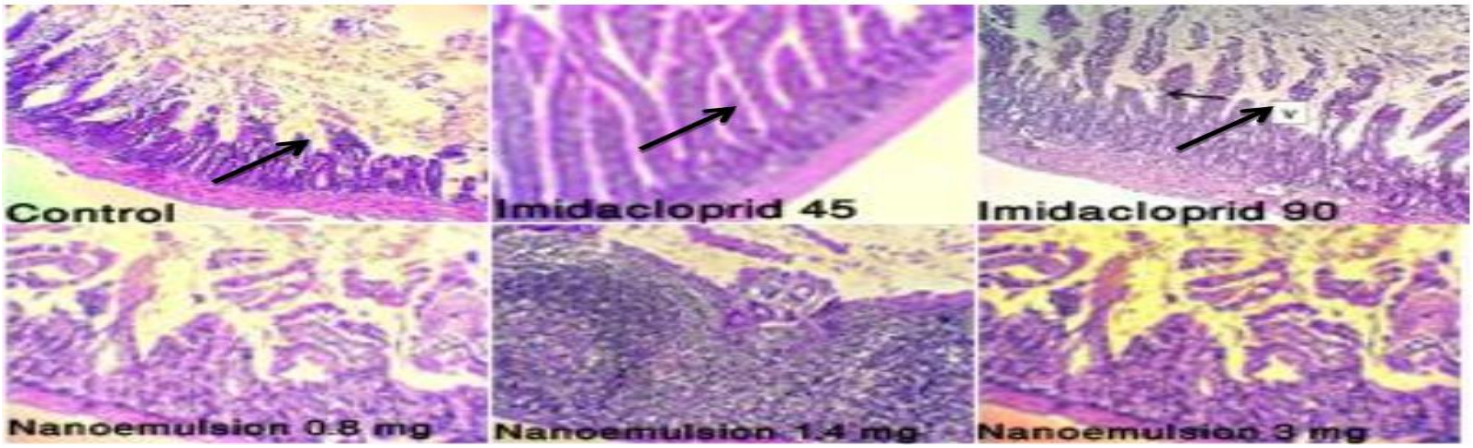

Figure 9: Histological examination of the intestine showed normal villous pattern, and minimal lamina propria inflammatory cells in control group (black arrow). Inflammatory cells with severe focal necrosis appeared in IMD nanoemulsion groups as a villous broadening (V), and lamina propriabut in mild form in IMD common preparation.

DISCUSSION: In the present study, Nano- imidacloprid was synthesized and characterized with zeta potential, FTIR, and electron microscope. Zeta potential is a stability-related value that measures the potential stability of the system and the repulsion or attraction magnitude of the electric charges at the interface of nano-emulsion droplets. A dividing line between stable and unstable aqueous dispersions is generally taken at either +30 or −30mV (Singare *et al.*, 2010). The Zeta potential of the system was found to be −32.8 mV, which indicated the desirable stability and negatively charged surface of nano-imidacloprid. The presence of a high negative charge could be due to the presence of anionic group present surfactant, and co-surfactant, used the preparation. Nephrotoxicity and hepatotoxicity are the potential complications of imidacloprid, which is widely used in crop production, and an assessment of its relative toxicity is important. We have investigated the possible effects of synthesized Nano-imidacloprid on rats' body weight, liver, and kidney functions. The acute oral toxicity study was conducted prior to the main experiment and the LD_{50} value was found to be 39mg/kg body wt. Our result shows that 21 days of oral exposure to high doses of commercial imidacloprid and different doses of Nano-imidacloprid produced significant toxic effects. Statistically significant reductions in body weight gain and feed consumption were noted in all doses. Changes in body weights have been used as an indicator of adverse effects of drugs and chemicals. A decrease in body weight gain in insecticides treated mice may be due to the effect of insecticide on the gastrointestinal tract resulting in decreased appetite and

absorption of nutrients whereas other workers explained reduced body weight gain as an indication of direct toxicity or stressogenic activity of these compounds. The reduction in body weight could also be attributed to the anorectic properties of insecticides. The transaminases (AST and ALT) are well-known enzymes used as a biomarker to predict possible toxicity. When the liver cell membrane is damaged, these different enzymes located in the hepatocyte cytosol are secreted in the blood (Ncibi *et al.*, 2008). Our current study reveals that oral administration of Nano IMD caused liver damage to rats as evidenced by significant increases in serum levels of AST, ALT, in all Groups when compared to the control group. The above findings were confirmed by histopathological changes in the liver under the intoxication effect of both forms of imidacloprid. of imidacloprid caused marked damage of the liver tissue in the form of hepatocytes hydropic degeneration and areas of centrilobular necrosis of hepatic cells. It has already been shown in previous studies that IMD induced elevation of hepatic enzymes. Thus, the observed elevation in these enzymes could be due to liver dysfunction attributed to the damaging effect of nano-imidacloprid on the liver cell membrane, which is in line with the study of Awad et al.[2] who found a good correlation between cell damage and the enzyme leakage. Supporting these findings (Lonare *et al.*, 2014) reported a marked elevation in AST, ALT, and ALP of IMD treated rats. Results are also by those of Balani *et al.* (2011) in a study of male White Leghorn (WLH) chicks treated with different concentrations of IMD. A study carried out by Vohra *et al.* (2014) on female albino rats following oral administration of two doses of IMD for 60 days revealed no significant increase in ALT, AST activities. The total protein is a common test to assess the toxicological nature of diverse chemicals. A decrease of total protein was observed in the present study following IMD treatment in Groups 2 and 3 and Nano imida (groups 4,5,6) when compared to the control group. This decrease can be caused by protein synthesis shortage as a result of liver dysfunction induced by the existence of IMD. Vohra *et al.* (2014) observed no significant change in total protein concentration after oral administration of IMI in calves and rats. Creatine found in muscle tissue and its catabolism to creatinine occurs at a steady rate. Severe kidney damage will lead to increased creatinine levels. In the present study, serum creatinine and blood urea nitrogen showed a significant increase in the nano-imidacloprid groups in comparison to control and commercial imidacloprid groups and this increase relates to renal failure. Serum creatinine and blood urea nitrogen (BUN) determine the glomerular filtration rate (GFR) improperly in renal failure. Serum creatinine and BUN have the potential to be a more precise marker for GFR. Similar results were reported in earlier studies in rats (Buege and Aust, 1978). In studying the genotoxic effects of imidacloprid by comet assay depends upon the fact that free radicals produced for pesticides and causes DNA damage (Singh et al., 2006; Zama et al., 2007). In these areas imidacloprid treated groups Showed an increase in the tail moment which indicator for the genotoxicity in the liver DNA. In comparison to commercial–imidcloprid, Nano-imidacloprid tends to exhibit higher toxicity. This was due to the improved dosage availability and dissolution rate of the active compound in Nano-imidcloprid which tend to cause toxicity even at a lower concentration (0.8mg/kg body weight). The higher toxicity exerted by the nano-imidacloprid was due to the nanometric colloidal form and higher surface area which facilitate higher penetration efficacy onto the rat tissues. In addition, the hydro-dispersive property of nano-materials improves the mobility and dosage availability of active compounds in the water matrix. Our findings are in agreement with Memarizadeh *et al.* (2014) as the Nano encapsulated form of imidaclorid exerted higher efficacy on Glyphodespyloalis larvae, comparative to its parental form. Similarly, Nano-permetrin tends to exert higher toxicity on the *Culex quinquefasciatus* Larvae than the parental-permethrin.

CONCLUSION: This study confirmed that imidacloprid conversion into nanoemulsion form increased its toxic and tissue damage effects, in addition to their severe DNA damage due to increasing the cell penetration power of the nanoemulsion form.

CONFLICT OF INTEREST: Authors have no conflict of interest.

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