Research Article

Rats Subchronically Exposed to Deltamethrin Nanoformulations Exhibited Less Genotoxicity Compared with those Exposed to Deltamethrin

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Abstract | The genotoxicological investigation of nanopesticides against a non-target model organism, the rat, will be a great way to learn more about how nanopesticides interact with the environmental and biological systems. Few studies on the genotoxicity of subchronic oral exposure to nano pesticides have been published. Male albino rats have been randomly divided into 4 identical groups. Group (I) had received only corn oil and acted as a control group. Group (II) had been given 3.855 mg/kg BW deltamethrin. Group (III) obtained deltamethrin loaded silica Nps at a dose of 8.795 mg/kg BW. The group (IV) obtained deltamethrin loaded chitosan Nps at a dose of 30.44 mg/kg BW. All remedies were administered by oral gavage 5 days a week for one month. The deltamethrin group showed a highly significant elevation in chromosome aberrations. Also, the ratio of polychromatic erythrocytes to normochromatic erythrocytes proved that deltamethrin had a stronger cytotoxic effect on the bone marrow cells than the CS/DM Nps and S/DM Nps groups. It was concluded that these innovative nanoformulations of DM were less toxic, which would lower health risks and boost application safety.

Keywords | Cytotoxic, Chromosome aberrations, Loaded, Nanopesticides, Safety

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INTRODUCTION

Pesticides are used to kill or repel pests. The widespread use of pesticides in agriculture has resulted in emergence of pesticide-resistant pests. Increased agricultural output necessitates increased pesticide use (Mostafalou and Abdollahi, 2013). Large amounts of potentially harmful substances have been released into the environment as a result of the extensive use of chemical pesticides in the food production and public health care, the majority of which are unspecific and hence could possibly target the human (Van and Pletschke, 2011). People can be exposed to pesticides on a frequent basis, for example, in the form of food contamination on the manufacturing line, but also in the home, at work, in hospitals, and in schools (Aprea, 2011).

The actual amount of pesticides which reaches the target organism is less than 0.1% of the applied amount. The remaining part was exposed to degradative processes and had bad effects on the non-target ones as fish and birds (Arias-Estévez et al., 2008).

Pyrethroids insecticides have appeared as a major family of

highly active insecticides due to low mammalian toxicity and high bio-efficacy comparable to organophosphorus and organochlorine. As a result, the exposure to organophosphorus pesticides dropped while pyrethroid exposures increased (El-Magd et al., 2011).

Deltamethrin (DM) is a synthetic pyrethroid type II, characterized by high insect control efficiency and low bioaccumulation in crops so, in most regions, DM had been the preferred insecticide (Köprücü et al., 2008). During DM metabolism, free radicals are generated leading to oxidative stress. These free radicals damaged the nucleic acids (Sharma et al., 2013). DM inhibited the mitotic index and induced chromosomal aberrations (Şekeroğlu et al., 2013).

Nanotechnology had progressed to the point where it had a significant impact on all aspects of human, animal, environmental, and industrial life. The addition of 5 ppm gold NPs to broiler chickens' drinking water on a weekly basis improved their growth performance and immune defence without changing the histological structures of their internal organs. On the other hand, adding of 15ppm gold NPs to drinking water caused significant blood oxidative stress damage, histopathological changes, up-regulation of Nrf 2,IL-6 gene expression, and DNA fragmentation in the broiler chicken immune organs, as well as a significant reduction in antibody titers against avian influenza viruses and Newcastle (Hassanen et al., 2020).

Chitosan, a linear copolymer, is the second most abundant polysaccharides in nature, comprising the horny substance in the exoskeletons of crabs, shrimp, and insects. Because some of their derivatives are proved to be non-toxic, biodegradable and biocompatible, they have been widely used in medical practice (Rinaudo, 2006). Chitosan NPs improved the antibacterial effect of Ag-NPs aganist *E. coli* as investigated by a marked decrease in both bacterial count and lesion score in broiler organs and reduce their toxicity in different organs. Also, chitosan NPs improved the body weight in broilers without leaving any silver residues in edible organs (Hassanen et al., 2021c). Mixing between chitosan and silver NPs in one nanocomposite (Ch-Ag NCs) showed the lowest bacterial count of methicillin-resistant Staphylococcus aureus in male albino Wistar rats in comparison with chitosan NPs and silver NPs (Hassanen et al., 2021c).

The toxicity of Ch-AgNPs was dose-dependent, and repeated dosing of rats with 50 mg/kg Ch-AgNPs resulted in severe toxicity. In renal and hepatic tissue, histopathological analysis revealed necrosis, apoptosis, cellular degradation, and congestion, as well as lymphocytic depletion with increased visible macrophages in the spleen. In this group, the greatest amounts of aspartate aminotransferase, alanine aminotransferase, and malondialdehyde were found, as well as the lowest levels of immunoglobulin G, M and reduced glutathione (Hassanen et al., 2019a).

Among variety of nanoparticles, the silica nanoparticles, have been intensively used for encapsulation of drugs (Ma et al., 2013). Avermectin, an insecticide was carried into porous hollow silica nanoparticles systhesized by sol-gel method with different capsule thicknesses of 5, 15, 30 and 45 nm. The loaded amount of avermectin decreased with the increase of shell thickness while protection against photo-degradation enhanced. In the same time the slowest release from the core shell observed in 45 nm (Li et al., 2006).

Nanotechnology can improve pesticide efficiency by controlling the release of the active ingredient through encapsulation in nanomaterials, to ensure sufficient amounts of the pesticide over a period of time, to obtain the maximum biological efficacy, and to minimize the unwanted effects (Tsuji, 2001), Increasing the delivery of hydrophilic pesticides and protecting the active ingredient against biodegradation (Kah and Hofmann, 2014).

The recent interest toward the nanopesticides usage in agriculture increases concerns about the potential genotoxic effect of nanopesticides on humans and non-target creatures. Thus, genotoxicity, ecotoxicity, and cytotoxicity evaluations are needed to fully comprehend the hazards associated with their use (Nagy et al., 2020). Nanopermethrin, one of nanopesticides was prepared by using solvent evaporation of oil in water microemulsion. Even though NP possesses a potent and selective larvicide for *Culexquinque fasciatus* than bulk permethrin (Anjali et al., 2010), the toxic effect on the animal or mammalian model remains unexplored.

Chromosomal abnormalities and increased micronuclei have been demonstrated in rat and mouse bone marrow cells (Chauhan et al., 2007). Free radicals attack DNA resulting in clastogenic and DNA damages (Jha, 2008). The *in vivo* studies for genotoxicity evaluation are an important tool for the chemical safety assessment, such as nano pesticides (Jain and Pandey, 2019). Rodents micronucleus (MN) assay is a component of quantitative risk assessments (Morita et al., 2011). The MN assay is one of the best *in vivo* cytogenetic assays due to the ability of micronuclei scoring and the easy to identify the newly formed erythrocytes (Hayashi, 2016).

There are scarce studies that evaluated the *subchronic* genotoxicity *of* nano pesticides *in vivo*. As a result, the aim of this work was to prepare chitosan and silica NPs,

coat DM with chitosan NPs, conjugate DM with silica NPs, measure zeta potential and the hydrodynamic size of the nanoformulations and to evaluate the sub chronic genotoxic effect of DM, S/DM Nps, and CS/DM Nps in rodents. This research represents the first sub chronic study of nano deltamethrin *in vivo*.

MATERIALS AND METHODS

Chemicals

Deltamethrin (98% pure) was obtained from El Naser Pharmaceutical Company (Egypt). Chitosan was purchased from Thermo Scientific. Colchicine, Sodium tripolyphosphate and tetraethyl orthosilicate were purchased from Sigma-Aldrich.

Animals

Sixty male albino Wister rats, weighing 0.150–0.170 kg were derived from the pharmaceutical department, laboratory animal unit, Faculty of Pharmacy, Beni-Suef University, Egypt. All animals procedures were conducted following the standards set forth guidelines for the care and use of experimental animals by the Animal Ethics Committee of Zoology Department in the college of Science at Beni-Suef University (Approval number is 021-137). All of the rats were kept in plastic cages (5 rats per cage) in a well-ventilated environment with 12 hours of light every day. They had been fed on dry commercial standard pellets throughout the trial, water was available at all times. They were given a two-week acclimatisation period.

Preparation of chitosan Nps (CS Nps)

CS Nps were produced by using the ionic gelation technique of sodium tripolyphosphate (TPP) with CS (Tığlı and Pulat, 2012). CS solution was formed by 1% (v/v) acetic acid. 1N NaOH elevated the pH to 4.6–4.8 then it was filtered. 4 mL of 1.5% wt/v TPP solution was then added (0.3 ml/min) to 10 ml of CS with magnetic stirring at 800 rpm at room temperature for 60 min, leading to spontaneous production of CS Nps. Nps were centrifugated for 30 min at 12,000 rpm to eliminate unreacted CS. CS Nps were washed by distilled water to remove any NaOH. The synthesized CS Nps were stored at 4°C and characterized (Da Silva et al., 2015).

Preparation of deltamethrin loaded CS Nps(CS/DM Nps) DM was loaded according to the method described by Servat-Medina et al. (2015). Before adding TPP solution, DM was added to the chitosan solution, and 1 percent Tween 80 was added in magnetic stirring for 60 minutes, followed by centrifugation at 30,000g for 45 min to separate non-entrapped DM. The purified Nps were freeze-dried.

Preparation of silica Nps

Silica Nps were formed by tetraethyl orthosilicate hydrolysis (TEOS) in ethanol, ammonia solution act as a catalyst for condensation (Wang et al., 2014). 50 milliters of ethanol (91%) and TEOS (9%) were sonicated, and 50 ml of NH3•H2O (18%) and deionized H2O (49.5%) added under strong stirring at room temperature for 2 hours. After centrifugation at 60,000 rpm for 10 minutes, the particles were washed twice with water and ethanol, followed by ultrasonication and centrifugation. To remove organic impurities, the particles were dried at 100–120 °C for 6 hours under ambient air before being calcined at 600 °C for 5 hours.

Loading silica Nps with DM(S/DM Nps)

DM loading was done by using the immersion loading method reported by Wen et al. (2005). 1.0 g of DM dissolved in 2.5 ml acetone and 0.25 g of silica Nps were added. The mixture is then stirred with magnetic stirring at room temperature with 400 rpm speed for 30 min. A white turbid suspension formed. The formed material was washed with 30% ethanol and centrifuged at 13000 × g, 5 °C for ten min then dried in air for 24 hr at 40 °C to produce a white powder.

Characterization of the nanoparticles using dynamic light scattering technique (DLS)

Particle size and the surface charge (zeta potential) were measured by Zetasizer (Malvern Instruments).

The sub chronic genotoxicity study of DM, S/DM Nps, and CS/DM Nps

Rats have been randomly divided into 4 identical groups (n=15). Group (I) had received only corn oil and acted as a control group. Group (II) had given 3.855 mg/kg BW deltamethrin(corresponding to1/10th median lethal dose (LD50) value: 38.55 mg/kg). Group (III) obtained S/DM Nps at a dose of 8.795 mg/kg BW (corresponding to 1/10th LD50 value: 87.95 mg/kg). The group (IV) obtained CS/DM Nps at a dose of 30.44 mg/kg BW (corresponding to1/10th LD50 value: 304.438 mg/kg). All remedies were administered by oral gavage 5 days a week for one month. Animals were anaesthetized with an intra-peritoneal injection of a 1:1 ketamine: xylazine combination (0.1 ml/100 gm. BW.) at the end of the study.

Chromosomal aberration assay

After 24 h from the last dose administration, An aqueous solution of colchicine (4 mg/kg body weight) was injected intraperitoneally 2 h prior to euthanization and decapitation. The bone marrow of the femur was excised with warm isotonic NaCL and incubated in 0.75 M KCl at 37°C for 30 min, then centrifuged at 1500 rpm for 10 min. The resulting pellet was fixed in a freshly prepared fixative

solution acetic acid/methanol (1:3) dropwise adding. The fixation step and recentrifugation were repeated twice. The fixed cells were resuspended in the fixative solution and dropped onto slides that have been chilled in 70% alcohol, air dried, and then stained on the next day in 10% buffered Giemsa (pH 6.8). One hundred well-spread metaphases were examined per rat. The classification of chromosomal aberrations was performed as described by Tice et al. (1987).

Micronucleus test

24 h after the last dose, the rats were euthanized by cervical dislocation to obtain the femur, the bone marrow flushed out with 1.5 mL fetal bovine serum (FBS) into an eppendorf tube. The resultant cell suspension was centrifuged at 1000 rpm for 10 min, the supernatant was discarded, and the pellet was washed with FBS then resuspend in a drop of FBS. The suspensions were uniformly spread on the slides and air-dried. The slides are then fixed in methanol for 3–5 min, and stand at room temperature overnight. The slides were stained with 1% May–Grunwald stock solution for 5 min then with May–Grunwald and phosphate buffer (1:1 v/v) for 5 min, rinsed with a distilled water and air dried. Then the slides stained with 5% Giemsa in PBS for 10 min, rinsed with a distilled water, and air-dried. The frequency and percentage of micronuclei were evaluated by scoring the slide under an oil immersion lens. 2000 erythrocytes (polychromatic and normochromatic) were scored for each rat (OECD, 2014).

Statistical analysis

The statistical analyses were calculated, using Statistical Package for Social Sciences (SPSS version 21.0, Inc., USA). The data were analyzed by using one-way ANOVA analysis and Duncan's multiple range tests. Values are the mean±SD (standard deviation) for 5 rats in each group. $P<0.05$ was in the accepted significance leve.

RESULTS

The hydrodynamic size of the nanoparticles (measured by DLS)

The result showed the size of nano-silica,which was used in this study, was 125.3 ± 8.86 nm while the nano-chitosan was 240.4±6.20 nm (Figure 1). The average size of silica loaded deltamethrin was 280.6 ± 8.23 nm while that of chitosan loaded deltamethrin was 561.3± 18.36 nm (Figure 1).

Zeta potential of the NPs

NPs obtained a negative Zeta potential values: Silica NPs (–19.5 mV), chitosan NPs (–10.2 mV), S/DM Nps (–22.1 mV), and CS/DM Nps (–11.4 mV) (Figure 2).

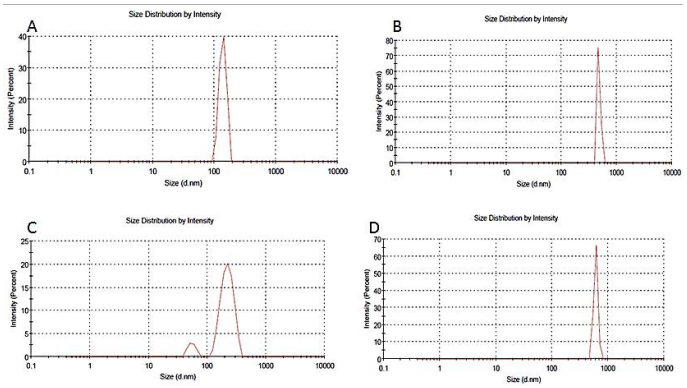

Figure 1: DLS measurements of particles size distributions after re-dispersion of silica powder (A), chitosan powder (B), silica loaded deltamethrin powder (C),and Chitosan loaded deltamethrin (D).

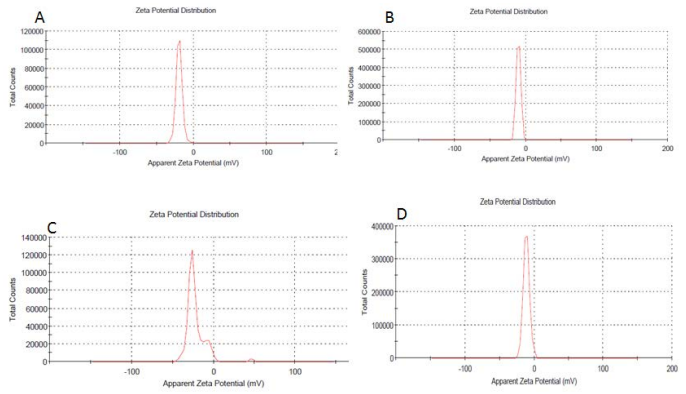

Figure 2: Zeta potential of silica NPs A, chitosan NPs B, S/DM Nps C, and CS/DM Nps D.

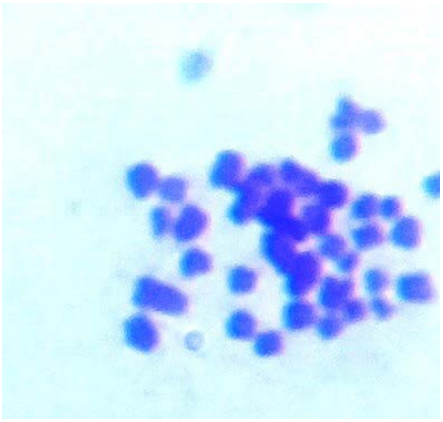

Figure 3: Metaphase spread of the bone marrow cells for the control rat.

Cytogenetic effects of DM, S/DM Nps and CS/DM Nps on rat bone marrow cells

Chromosomal aberrations in bone marrow

We studied the chromosome aberrations to assess the genotoxicity induced by DM, S/DM Nps, and CS/DM Nps (Table 1). The structural chromosomal aberrations were observed, such as rings, deletions, end to end associations, breaks, centric separations, and stickiness (Figures 3, 4, 5 and 6). Data showed that conjugation of DM with

silica or chitosan NPs did not influence the appearance of chromosomal end to end associations. There was no significance between DM, CS/DM Nps, and S/DM Nps in the number of chromosomal end to end associations. The highest average number of deletions was detected as a result of the exposure to S/DM Nps and differed significantly from the DM group (p=0.001). Although treatments with DM and CS/DM Nps were not significantly different in the average number of centric separations (p=0.10) and showed a higher number than S/DM Nps. The DM group showed a significant decrease in the average number of sticky chromosomes than S/DM Nps and CS/DM Nps (p=0.000 and p=0.002). On the other hand, rings revealed the highest values in CS/DM Nps. Meanwhile, the frequency of break was highly elevated due to DM as compared with S/DM Nps, CS/DM Nps, and control. The result revealed that numerical aberrations were found to be statistically significant in all treatments. The hypoploidy frequency was more prominent in the S/DM Nps group while hyperploidy was more prominent in the DM group and significantly differed from CS/DM Nps (p=0.034).

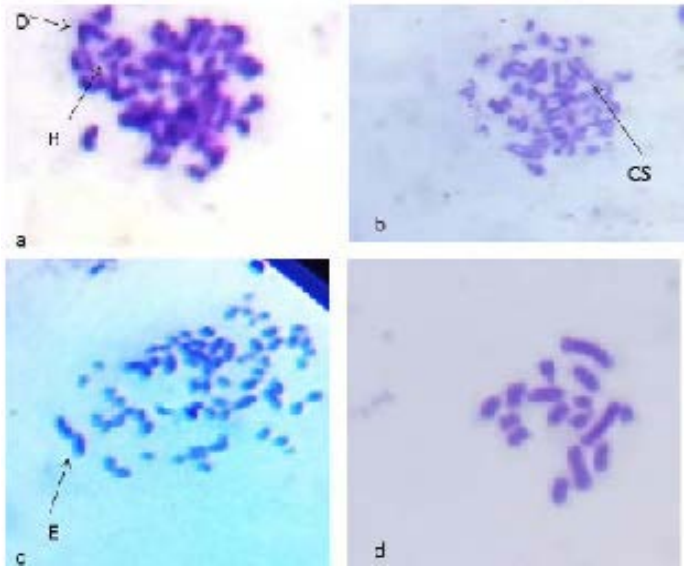

Figure 4: Metaphase spread from the rat bone marrow cells; after 30 days of deltamethrin administration (a): metaphase spread showing break (B) and deletion (D). (b): metaphase spread showing centric separation (cs). (c): metaphase spread showing end to end association (E) and hyperploidy.(d): metaphase spread showing hypoploidy.

Micronucleus assay

In the control group, 13.33 micronucleated PCE cells were obtained among 2000 examined cells, which represent 0.33%, while in the DM treatment group, 69.66 MnPCEs were counted which represent 1.74%. 19 and 49.33 MnPCEs were obtained in S/DM Nps and CS/DM Nps, respectively which represent .47% and 1.23% (Table 2). The obtained results showed that DM showed a significant increase in the frequency of micronucleated PCE compared to control group. The lowest number of micronucleated PCE cells in insecticide treatments was found in S/DM Nps (Figure 8). The DM group showed a significant increase in the MnPCEs than CS/DM Nps (p=0.008) (Figure 7).

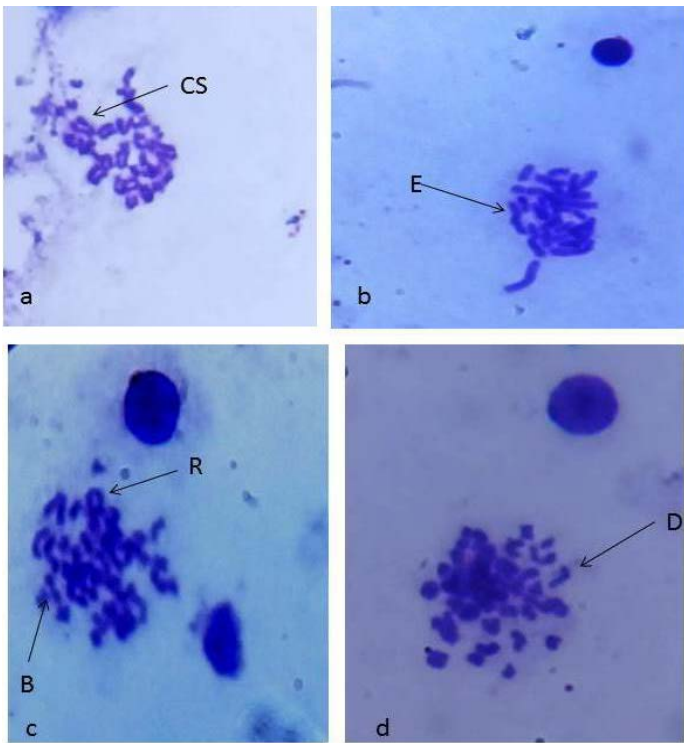

Figure 5: Metaphase spread from the rat bone marrow cells; after 30 days of S/DM Nps administration (a): metaphase spread showing centric separation (cs). (b): metaphase spread showing end-to-end association (E) and hypoploidy (c): metaphase spread showing break (B) and ring(R).(d):metaphase spread showing deletion (D).

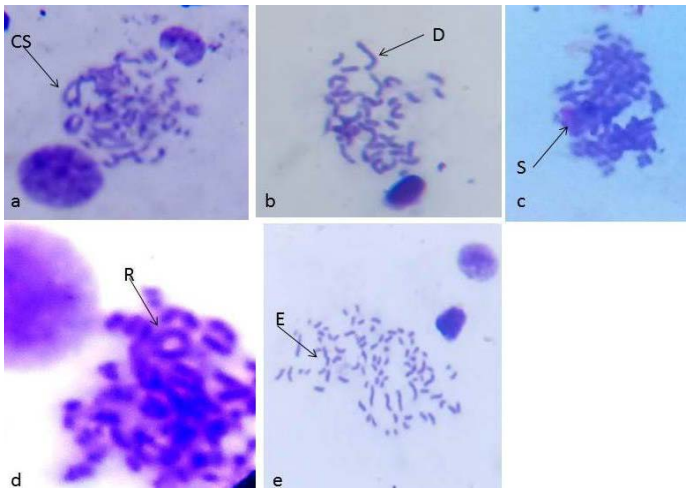

Figure 6: Metaphase spread from the rat bone marrow cells; after 30 days of CS/DM Nps administration (a): metaphase spread showing centric separation (cs). (b): metaphase spread showing deletion (D). (c): metaphase spread showing stickness (S). (d): metaphase spread showing ring(R).(e): metaphase spreads showing end to end association (E) and hyperploidy.

Table 1: The chromosomal aberrations observed in bone marrow cells of male rat treated with DM, S/DM Nps,and CS/DM Nps.

	Structural aberrations						Numerical aberrations	
Groups	End to end associations	Deletions	Centric separation	Sticky chromosomes	Ring	Breaks	Hypoploidy	Hyperploidy
Control	00 ± 0[a]	00 ± 0[a]	00 ± 0[a]	00 ± 0[a]	00 ± 0[a]	00 ± 0[a]	6.66 ± 2.88[a]	.66±.57[a]
DM	6.66 ± 1.52[b]	2.66 ± 1.15[ab]	12 ± 3[c]	00 ± 0[a]	00 ± 0[a]	1±.57[b]	42.33 ±2.08[b]	46±1[c]
S/DM Nps	8±2[b]	11.33 ±3.05[c]	3.66 ±.57[b]	4.66 ± 1.52[b]	.66±.57[a]	.57 ±.33[a]	91.33 ±1.52[c]	00±0[a]
CS/DM Nps	7.33±2.08[b]	4 ± 2[b]	9.66 ±.57[c]	7 ± 2[b]	6 ±1[b]	00 ± 0[a]	46 ± 3.60[b]	43.00±2.64[b]

Metaphase score of chromosomal aberrations (100 well spread metaphase for each rat). Values are the mean±SD for 5 rats in each group.[abc] The means within the same column and bearing different superscripts are significantly different at $P<0.05$.

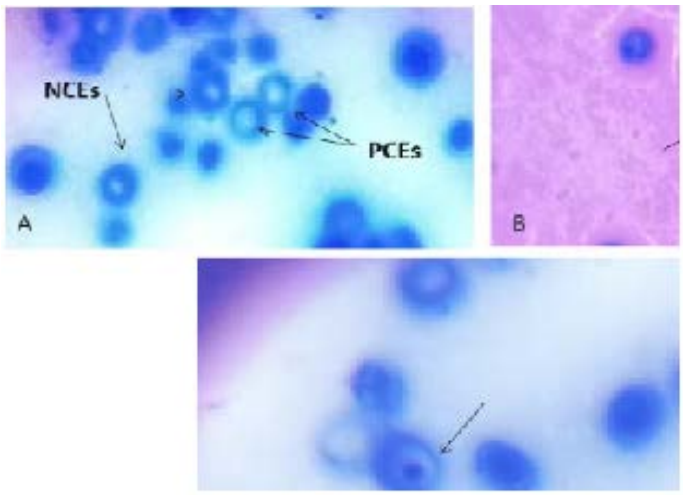

Figure 7: The different forms for the micronuclei in polychromatic erythrocytes of rats administered deltamethrin.

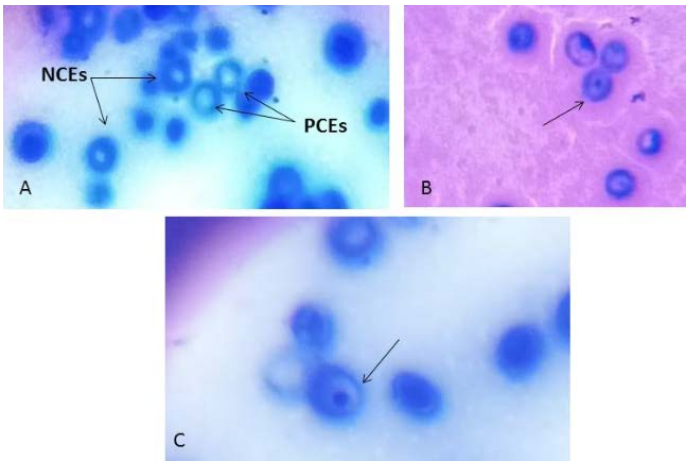

Figure 8: Microphotographs of polychromatic erythrocytes and normochromatic erythrocytes in control rats (A), polychromatic erythrocyte micronucleus in S/DM Nps administered rats(B) and CS/DM Nps (C).

Polychromatic erythrocytes/Normochromatic erythrocytes (PCEs/ NCEs) ratio

The PCE/NCE ratio in the DM and CS/DM Nps groups was significantly decreased (P<0.001) when compared to the control group. The S/DM Nps group showed a significant decrease than the control (p=0.008) and a significant increase than the DM(P<0.001) Table 2.

Table 2: Effects of deltamethrin, S/DM Nps and CS/DM Nps sub chronic administered on the frequency of *micronucleated polychromatic erythrocytes* (MnPCEs) and polychromatic to normochromatic erythrocytes (PCEs/NCEs) ratio.

Groups	MnPCEs/ 2000PCEs	PCEs/ NCEs	MnPCEs percentage %
Control	13.33 ± 3.51[a]	59.66 ± 4.50[c]	.33 ± .08[a]
DM	69.66 ±9.50[c]	8.66 ±3.78[a]	1.74 ± .23[c]
S/DM Nps	19 ± 4.00[a]	44 ±3[b]	.47 ± .10[a]
CS/DM Nps	49.33 ± 9.01[b]	12.66 ±8.62[a]	1.23 ± .22[b]

Values are the mean±SD for 5 rats in each group.[abc] The means within the same column and bearing different superscripts are significantly different at $P<0.05$.

DISCUSSION

The toxicity evaluation of nanoparticles prior to clinical and biological uses has received a lot of attention. CuO-NPs used as a feed additives to broiler chickens. Weekly oral intake of 15-mg CuO-NPs through the life of the chicks caused a significant increases in DNA fragmentation percentage due to oxidative stress (Morsy et al., 2011).

CuO-NPs cause neurodegeneration and neurobehavioral toxicity in the brain of male albino Wistar rats by causing apoptosis, inflammation, DNA damage, and oxidative stress by releasing different mediators from astrocytes and microglia (Hassanen et al., 2021a). CuO-NPs injected rats had higher levels of AST, ALT, creatinine and blood urea nitrogen, altered oxidant–antioxidant balance, severe pathological alterations in kidney and liver tissues, and overexpression of both nuclear factor kappa B protein (NF-B) and caspase-3 associated with downregulation of Bcl2 gene and upregulation of Bax gene. All of the aforesaid toxicological parameters were improved by pomegranate juice (Hassanen et al., 2019b).

The use of ZnO-NP cream on the skin of lead-intoxicated rats mitigated lead toxicity in body organs.This protective mechanism can be linked to ZnO-NPs' capability to inhibit lead ion absorption through the skin, as well as their antioxidant and anti-apoptotic properties (Hassanen et al., 2021b).

Nanopesticides are not only in advanced stages of development, but they are also beginning to enter the market. For human health risk evaluations, the regulatory and industry agencies require a unified and comprehensive framework and guideline. It will be essential to design strategies to appropriately address the regulatory requirements for the emerging nanopesticides (Kah et al., 2021).

Pesticides such as organophosphates and pyrethroids disrupt the oxidative homeostasis through the generation of free radicals in cells like O2-, H2O2, and •OH. These radicals damage biological macromolecules such as RNA, DNA, and DNA repair proteins (Zepeda-Arce et al., 2017).

The results reported a significant increase in chromosome aberrations in the DM group over the S/DM Nps and CS/DM Nps groups. Similarly, Ismail and Mohamed (2012) demonstrated a significant increase in chromosome aberrations in bone marrow in rats treated with DM. Moreover, fragments, chromosome and chromatid gaps, chromatid breaks, and chromosome breaks were noted in rats with cyhalothrin after 30 days (Dinesh Sharma et al., 2010). The free-radicals originated from exposure to DM (Rodríguez et al., 2016), oxidize the DNA molecule and lead to elevated chromosomal aberrations. Chromosome aberrations are probably due to the interaction between the DNA molecule and insecticides (Şekeroğlu et al., 2013). DM induced structurally chromosomal aberrations in bone marrow cells that could be due to the decrease in the cleavage enzyme level, thus the cell failed to make chromosomal segregation (Wilstermann and Osheroff, 2005). In the Allium cepa test, nanopermethrin treatment of 0.13 mg/L showed a mitotic index of 52.0% and chromosomal aberration of 0.2%, which was not significant from the control. A significant difference was reported in 0.13 mg/L permethrin exposure as compared to control (mitotic index of 46.8 and 55.03% and chromosomal aberration of 0.6 and 0 %, respectively) (Suresh et al., 2013). The genotoxic effect of CS/DM Nps and S/DM Nps may be mainly due to the deltamethrin content, as the action of nanopesticide is mainly due to the active ingredient, i.e. deltamethrin (Mishra et al., 2017). At the same time, orally administreted chitosan Nps in packaged food, did not show signs of genotoxicity (De Lima et al., 2010). Oral intake in the dose range of 1–15 g/kg/day for up to 3 months has led to minimum clinical signs in both mouse and rat models (Baldrick, 2010). The data obtained here is similar to that in the paper, which showed that the unwanted effect of the herbicide Paraquat on human and animal health and the environment was reduced by encapsulation with chitosan NPs. As evaluated by the Allium cepa chromosome aberration test, the toxicity of the loaded paraquat was less than the pure one (Grillo et al., 2014).

Anucleated cytoplasm of polychromatic erythrocytes represents abnormal development of the erythrocytes (Jain and Pandey, 2019) caused by DM as in the normal state the main nucleus is extruded. The appearance of a small additional nucleus represents chromosomal damage and the increase in the frequency of MN PCEs is an indicator of genotoxicity (Jain and Pandey, 2019). The CS/DM Nps showed a less genotoxic effect in bone marrow than DM, while S/DM Nps did not report any effect. This may be due to that nano-pesticides release the active ingredient in a gradual pattern and conserve it against the environmental degradation (Kah and Hofmann, 2014). Simultaneously, no toxicologically significant changes in mortality, clinical signs, body weight, food consumption, necropsy findings, or organ weights were observed when silica NPs were administered orally to Sprague-Dawley rats for 3 months at doses of 166.7, 500, and 1,500 mg/(kg• BW•day) (Liang et al., 2018).

The decline in PCEs/NCEs ratio in DM and CS/DM Nps groups indicated that they had a strong cytotoxic effect on the bone marrow cells. In a study, after 4 weeks of deltamethrin (7.2 mg/kg b.w.) treatment, rats showed a rise in the hepatic LPO level and a reduction in CAT, GPx, and SOD activities followed by a significant increase in the bone marrow micronucleus frequency (Ncir et al., 2016).These abnormalities suggested that DM can induce oxidative and genotoxic damage *in vivo*.

CONCLUSIONS AND RECOMMENDATIONS

The study confirms that DM and its nanometric forms have an accumulative effect on the induction of chromosomal damage in male albino rats. The genotoxic effect of DM was much pronounced than CS/DM Nps and S/DM Nps, as evident from the observed results. As a result, extreme caution should be exercised when employing these nano insticides, particularly CS/DM Nps. When compared to free DM, these innovative nanoformulations of DM were less toxic, which would lower health risks and boost application safety. Future research on the toxicological potential of nano insecticides on health could be helped by our findings.

NOVELTY STATEMENT

This research represents the first genotoxicological investigation of the novel deltamethrin nanoformulations (deltamethrin loaded chitosan Nps and deltamethrin loaded silica Nps) *in vivo*.

AUTHOR'S CONTRIBUTION

A.G is the principal author and participated in the design of the study. w.A and A.Kh helped to draft the manuscript. H.M participated in the design of methodology.

Conflict of interest

The authors have declared no conflict of interest.

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