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Effects of the Nano-Profenofos Pesticide on the Chromosomes of Albino Rats

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Abstract

Background: **The management of insect pests through the formulations of nanomaterials-based insecticides is not uncommon recently. Traditional strategies used in agriculture are insufficient, and the application of chemical pesticides has adverse effects on animals and human beings apart from the decline in soil fertility. Therefore, nanotechnology would provide green and efficient alternatives for the management of insect pests in agriculture without harming nature. However, the disadvantages of nanotechnology are possible in both occupational and residential environments.** ***Aim:*** **This study aimed at the evaluation of the genotoxic effect of the pesticide Profenofos (organophosphorus) and its nanoparticles in rats. This was assessed by the study of chromosomal aberrations.** ***Materials and Methods*****: Healthy male rats were orally fed with different doses (1/20 and 1/40 of the LD_{50}) of Profenofos and nano-Profenofos in multi doses for three successive days at two different time intervals (24 hours and one week) post-treatment.** ***Results:*** **The data revealed a significant increase in the number of chromosomal abnormalities in both pesticide-treated animals compared to their controls. The maximum genotoxic effect was induced by the nano-Profenofos pesticide.** ***Conclusion:*** **The results obtained in this study suggest that nano-pesticides may have higher genotoxic and biological effects than their regular pesticides.**

Keywords: Organophosphorus pesticides, Nanoparticles, Genotoxicity, Chromosomal aberrations

Introduction

Nanotechnology is a promising field of interdisciplinary research. It opens a wide array of opportunities in various fields like insecticides, pharmaceuticals, electronics, and agriculture. Nano-pesticides appear as an alternative because they can be used as 'smart delivery systems' for the release of pesticides in a timely controlled manner. This would reduce the risk of environmental pollution and its associated hazards[(1)]. Nano-pesticides offer advantages to producers to achieve economic benefits. The longevity of biological activities of the nano-pesticides compared to other pesticides reduces the amount of pesticide to be used[(2)]. On the other side, disadvantages of nano-technology[(3,4)] may include the ingestion of nano-pesticides that could be problematic in both occupational and residential settings. This study was designed to assess and compare the genotoxic effect of regular Profenofos (organo-

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phosphorous pesticide) and its nanoparticles in mammals. Foodstuffs contaminated with farm chemicals like fertilizers and pesticides are among the potent generators of free radicals like reactive oxygen species (ROS). Harmful effects of ROS on the cells are most often like damage of DNA, oxidations of polyde-saturated fatty acids in lipids, oxidations of amino acids in proteins, and oxidatively inactivate specific enzymes by oxidation of co-factors. Free radicals cause many human diseases like cancer, Alzheimer's disease, cardiac reperfusion abnormalities, kidney disease, fibrosis, etc.[5].

Material and Methods

1- Animals and treatment

A group of male albino rats (*Rattus norvegicus*) were obtained from the animal house of the Faculty of Veterinary Medicine, Zagazig University, Egypt having an average age of 2.5 - 3 months and the average of their weight was 150 - 200 gm. The animals were housed in plastic cages and supplied with enough food (standard pellets) and water and observed daily for a period of two weeks at least before any experimental action for acclimatization. They were classified as the following:

- *First group:* rats were given 1.0 ml of oil orally by oro-gastric tube for 3 successive days and served as control.
- *Second group:* rats were subdivided into many subgroups, with 5 rats in each subgroup. Each rat of these subgroups receives doses of 1/20 and 1/40 of LD50 of Profenofos and nano-Profenofos orally dissolved in corn oil by oro-gastric tube for 3 continuous days then the treated animals and their controls were sacrificed after 24h. and after one week of administration.

2- Chromosomal aberrations test

Cytogenetic analysis of chromosomal preparations was made from the femoral bone marrow cells and the technique was carried out according to Yosida and Amano[6]. Briefly, bone marrow cells were exposed to hypotonic treatment before fixation and dropped on clean slides then stained with Giemsa prior to microscopic analysis. For each animal, 50 chromosomal spreads were examined with light microscopy at 100X.

Results

The average percentages of total chromosomal aberrations from bone marrow cells of rats administrated orally1/20 and 1/40 LD_{50} of Profenofos and nano-Profenofos were 42.4% & 32.4% respectively for Profenofos and 46.8 %& 40.4% respectively for nano-Profenofos which is found to be highly significant after 24h post-treatment compared with that of their control group which was 14.8%. Moreover, the average percentages of the total aberrations of the dose of 1/20 and 1/40 of profenofos and nano-profenofos after a week post-treatment were 37.2% & 29.6% respectively for profenofos and 38% & 28% respectively for nano-Profenofos which is found to be also highly significant compared with that of the control (Table 1) & (Figure 1). The examination of metaphase spreads from the bone marrow of the control animals demonstrated that a few of these metaphases exhibited some numerical and structural aberrations (Figure 2). The total chromosomal aberrations from bone marrow cells of rats administrated orally1/20 and1/40 LD_{50} of Profenofos and nano-Profenofos was found to be highly significant after all time intervals post-treatment in which numerical aberrations were more than structural aberrations and the predominant structural aberrations were ex-

change figure and segregated chromosomes (Figure 3). Furthermore, comparing the effect of the doses 1/20 of LD_{50} as well as 1/40 of LD_{50} of Profenofos and nano-Profenofos revealed no significant difference in inducing chromosomal aberrations after 24h.

Table 1: Comparison between the percentages of chromosomal aberrations from bone marrow cells of male rats orally administrated with 1/20& 1/40 LD_{50} of Profenofos and Nano-Profenofos.

No. of rats	**Control***	**1| 20-24h***	**1| 20-24h NP***	**1| 20-week***	**1| 20-week NP***	**1| 40-24h***	**1| 40-24h NP***	**1| 40-week***	**1| 40-week NP***
%	14.8	42.4	46.8	37.2	38	32.4	40.4	29.6	28
P	----	0.0007***	0.0002***	0.0000**	0.0043**	0.0053**	0.0036**	0.0046**	0.0012**
S.D.	±1.82	±2.59	±1.67	±2.89	±2.65	±2.59	±2.39	±2.28	±2

Data represent mean values ± S.D. of the results obtained from 5 animals in each group.
***: significant at p < 0.01 when comparing the difference between each treated group and the control*
****: significant at p < 0.001. When comparing groups treated with Profenofos and Nano Profenofos (NP) no significant difference was observed (Student T Test).*
**: total chromosomal aberration for nano Profenofos (%)*

Discussion

In the present study, the structural chromosomal aberrations were more prominent than numerical aberrations after treatment with Profenofos and nano-Profenofos. The data obtained were statistically significant at both time intervals (24h and 1 week) post-treatment. It is possible that there are specific chromosomes affected by these pesticides and induced dysfunction in the centromere of chromosomes at the anaphase stage and/or pesticides' effect on spindle fibers by reaction with its protein[7]. Organophosphorus compounds were shown to possess alkylating properties to DNA and protein, leading to DNA damage. Profenofos is an alkylating agent that chemically alters DNA and inhibits DNA replication leading to the induction of chromosomal aberrations in the cells. Organophosphate toxicity may be attributed to the generation of reactive oxygen species and free radicals that can damage DNA through the oxidation of DNA bases or through covalent binding to DNA resulting in strand breaks and cross-linking [8-12]. Chromosome damage and mutagenicity are well-known effects caused by different chemical and physical agents. Sub-lethal proportions of agents that are mutagenic and carcinogenic to mammals interact with cellular DNA and are extremely potent in inducing chromosome damage, which may be detected cytologically. The results suggest the possibility of induction of DNA breakage and/or alkali-labile sites (ALS) and various species of oxidized purines and pyrimidines[13]. Damage to cellular DNA by lipid peroxidation plays a major role in cell injury and altered cell functions leading to apoptosis[14]. Thus, the role of oxidative stress in induced DNA damage cannot be excluded. The role of reactive oxygen species (ROS) in the production of DNA single-strand breaks (SSBs) is well known[15]. ROS interacts with biological molecules and disrupts the normal synthesis and repair of DNA.

Profenofos cause DNA damage and cytotoxicity[16] and they may induce in vivo and in vitro generation of reactive oxygen species leading to oxidative stress[17]. The non-significant difference (in some samples the effect was higher in the case of Nano-Profenofos) in the present study between the effect of the regular Profenofos and its nanoparticles suggest the incomplete safety of the nano pesticides and the benefit/risk assessment of such compounds are necessary before approving these pesticides for large scale use because they have higher genotoxic effects on the human. Searching for safe alternatives is highly recommended.

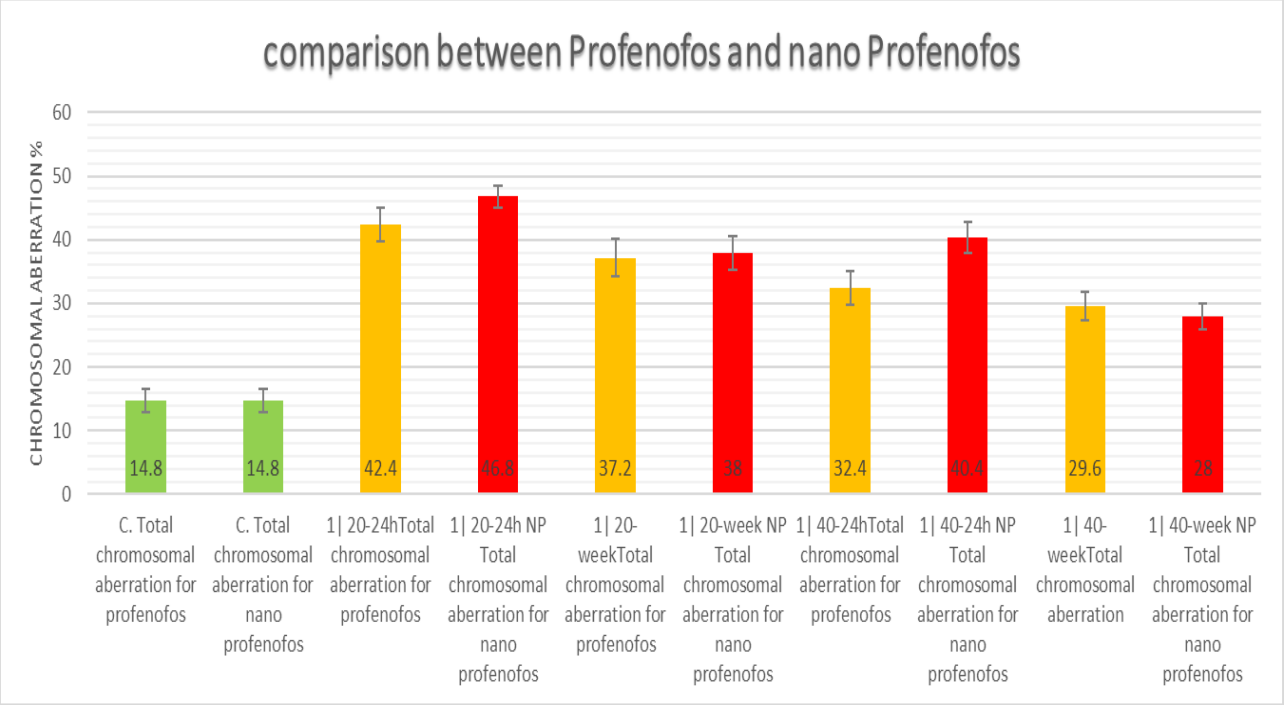

Figure 1: Comparison between the percentages of chromosomal aberrations from bone marrow cells of male rats administrated with 1/20& 1/40 LD_{50} of Profenofos and nano-Profenofos after 24h and after one week

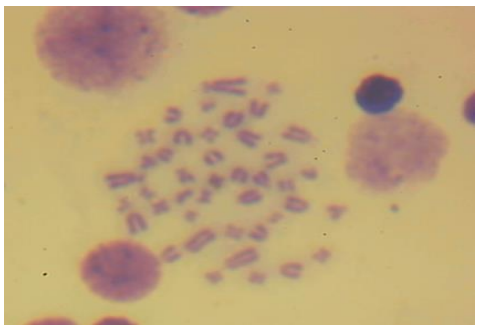

Figure 2: Metaphase chromosomes from bone marrow cells of the untreated male albino rats (control) illustrating normal chromosomes

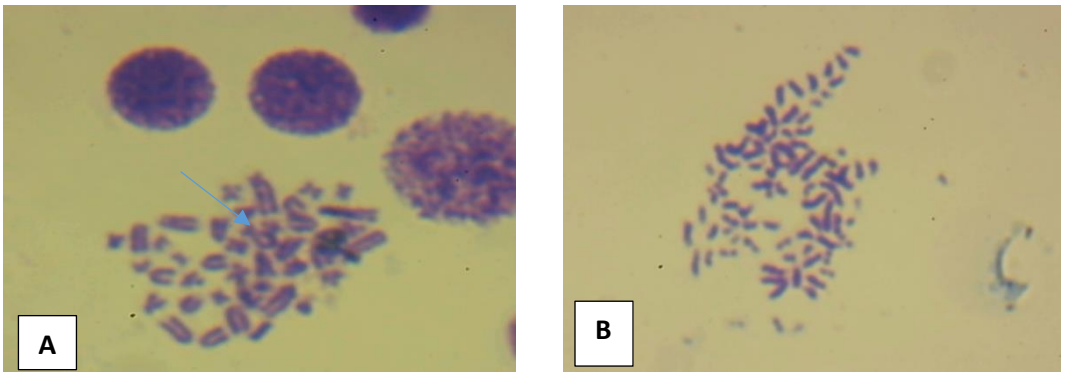

Figure 3: Effect of Profenofos and nano-Profenofos on metaphase chromosomes from bone marrow cells of the treated albino rats after oral administration with the pesticides illustrating chromosomal aberrations **A:** dicentric chromosome, **B:** segregated chromosomes

Conclusion

Profenofos is one of the most used organophosphorus insecticides on field crops, vegetables, and fruit crops for controlling the infestation pests of these crops. The results obtained in this study suggest that nano-pesticides may have higher genotoxic and biological effects than their regular pesticides. This research can be applied to human beings because this present study provides minor information on profenofos risk assessment to human consumers. However, additional risk assessment studies of profenofos pesticides are needed to fully clarify their risk and more safety studies and measurements are urgently needed for the risk/benefit assessment when using pesticides and chemicals in the environment.

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