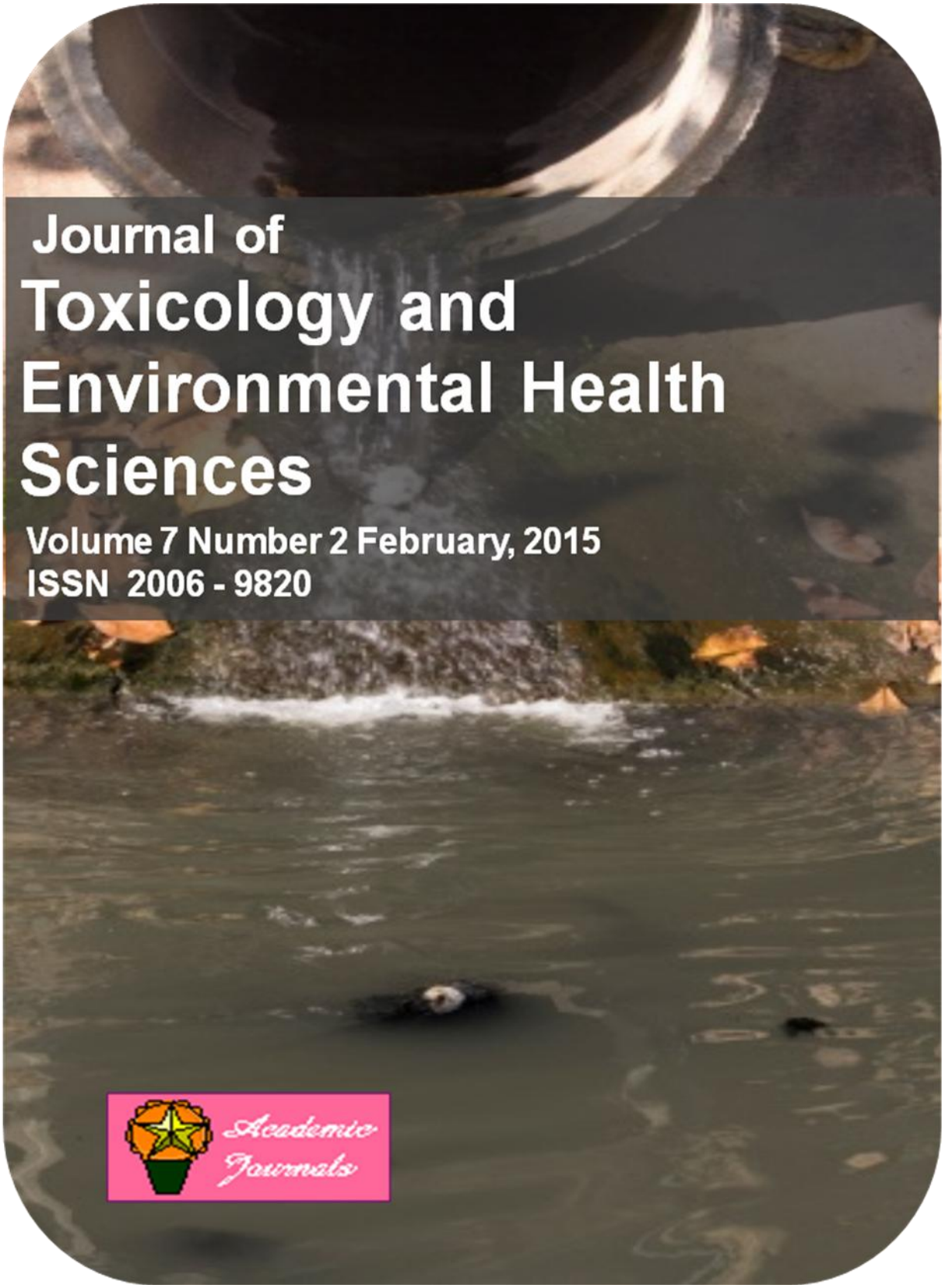

Journal of Toxicology and Environmental Health Sciences

Volume 7 Number 2 February, 2015
ISSN 2006 - 9820

Academic Journals

ABOUT JTEHS

The **Journal of Toxicology and Environmental Health Sciences (JTEHS)** is published monthly (one volume per year) by Academic Journals.

The **Journal of Toxicology and Environmental Health Sciences (JTEHS)** is an open access journal that provides rapid publication (monthly) of articles in all areas of the subject such as toxic genomics, enzyme inhibition, drug overdose, Children's Environmental Exposure Research Study etc.

The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JTEHS are peer-reviewed.

Submission of Manuscript

Submit manuscripts as e-mail attachment to the Editorial Office at: jtehs@academicjournals.org. A manuscript number will be mailed to the corresponding author shortly after submission.

The Journal of Toxicology and Environmental Health Sciences (JTEHS) will only accept manuscripts submitted as e-mail attachments.

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author.

Editors

Dr. Hazem Mohammed Ebraheem Shaheen
Department of Pharmacology, Faculty of
Veterinary Medicine, Damanhur University,
Behera – Dalangat – Elbostan,
Egypt

Dr. Jianbo Xiao
College of Life & Environment Science,
Shanghai Normal University 100 Guilin Rd,
Shanghai 200234,
PR China

Dr. Adriana Maria Neghina
Victor Babes University of Medicine and Pharmacy
Biochemistry Department
2 Eftimie Murgu Square
RO - 300041, Timisoara
Romania

Dr. Rouabhi Rachid
Biology Department
University of Tebessa 12000.
Algeria.

Prof. YongXun Pang
Endemic center, Harbin Medical University 157 BaoJian Road,
NanGang District, Harbin, P. R.
China

Dr.M.Mahadeva Swamy
Mysore – 570 006, Karnataka,
India

Dr. Shashank Shah
"40/29 Bhonde Colony, 14 Shwe Off Karve Road,
Erandwane,
Pune, Maharastra,
India

Dr. Necati Celik
Karadeniz Technical University, Dept. of Phys. 61080
Trabzon,
Turkey

Prof. Yangfeng Wu
"Suite B1302, No 6, Zhichunlu Rd., Haidian District,
Beijing, 100088,
China

Dr. Ashim Kumar Biswas
Department of Livestock Products Technology, COVS,
Ludhiana- 141004 (Punjab)
India

Dr. Ilia Yarmoshenko
Institute of Industrial Ecology of
Ural Branch of Russian Academy of Sciences
620219 S. Kovalevskoy Str., 20, Ekaterinburg,
Russia

Dr. Şifa Türkoğlu
Cumhuriyet University, Faculty of Art and Science,
Deparment of Biology, Sivas,
Turkey

Dr. Juan Antonio Riesco Miranda
Pneumology Department. San Pedro
Alcantara Hospital Cáceres
Spain

Dr. Norazmir Md Nor
Department of Nutrition & Dietetics
Faculty of Health Sciences MARA University of
Technology Puncak Alam Campus42300
Puncak Alam Selangor,
Malaysia

Dr. Helal Ragab Moussa
Bahnay, Al-bagour, Menoufia,
Egypt

Prof. Dr. Mamdouh Moawad Ali
33 El-Tahrir Street, Dokki 12622, Cairo,
Egypt

Reza Hosseinzadeh
Shahid Beheshty Ave., Urmia University,
Jahad-E-Daneshgahi, P. O. Box No. 165, Urmia,
Iran

Moustafa Hossein El-Naggar
Biological Sciences, Faculty of Science,
King Abdulaziz University,
Jeddah, KSA

Hasan TÜRKEZ
Division of Molecular Biology and Genetics,
Faculty of Science,
Erzurum Technical University, Erzurum,
Turkey

Journal of Toxicology and Environmental Health Sciences

Table of Contents: Volume 7 Number 2 February, 2015

ARTICLES

Research Articles

academicJournals

Vol. 7(2), pp. 9-17, February 2015
DOI: 10.5897/JTEHS2015.0330
Article Number: 48A505A50521
ISSN 2006-9820
Copyright © 2015
Author(s) retain the copyright of this article
http://www.academicjournals.org/JTEHS

Journal of Toxicology and Environmental Health Sciences

Full Length Research Paper

Exposure to ZnO-NPs enhanced gut- associated microbial activity in *Eisenia fetida*

Shruti Gupta, Tanuja Kushwah and Shweta Yadav*

Department of Zoology, School of Biological Sciences, Dr. Harisingh Gour Central University, Sagar-470003, MP, India.

Received 18 January, 2015; Accepted 2 February, 2015

With advent of the nanotechnology era, the environmental risk has continuously been receiving engineered nanomaterials, as well as their derivatives. Our current understanding of the potential impact of nanomaterials and their effect on soil organism is limited. The present study fills the gap between effect of manufactured nanomaterials (NPs) and their available natural scavengers. In the study, earthworm *Eisenia fetida (EW),* which occupies 60 to 80% of the total biomass and well known for its contribution to cellulolytic degradation of organic wastes, was exposed to ZnO-NPs. Findings suggests that *E. fetida* can survive even at high exposure of ZnO-NPs (10 mg/kg) and can exhibit increase in bio-accumulation of Zn content in its body tissue with decreased NPs. Exposure of 35 and 10 nm ≥3.5 mg/kg sized NPs showed an increase in cellulase activity by 38 to 41%. This increase in cellulolytic activity in EWs' gut may also be helpful in the bioconversion of lignocelluloses waste. Eighteen strains of cellulose hydrolytic bacteria capable of producing cellulase were obtained from the guts of EWs exposed to ZnO-NPs. The results of biochemical and 16SrRNA gene sequence examinations showed that six strains belongs to *Bacillus* sp.; five strains belongs to the sublines of *Bacillus* and others belongs to the *Pseudomonas* sp. The study advocates the application of ZnO-NPs enhance gut-associated microbial activity.

Key words: Cellulose hydrolytic bacteria, ZnO-NPs, *E.fetida,* Gut -flora**.**

INTRODUCTION

Manufactured nanoparticles have a wide range of application having unique preparation as compared with their bulk counterparts (Nel et al., 2006). Nano-forms of metals, metal oxides, carbon-based materials and biopolymers are being used in several applications. Zinc oxide nanoparticles (ZnO-NPs) are one of the most abundantly used nanomaterials in cosmetics and sunscreens as they efficiently absorb ultraviolet (UV) light and also do not scatter visible light. This makes ZnO-NPs more transparent, aesthetically compared to their bulk counterpart

*Corresponding author. E-mail: kmshweta3@yahoo.com.
Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

(Schilling et al., 2010). They are also being used in the food industry as additives and packaging due to their antimicrobial properties (Gerloff et al., 2009; Jin et al., 2009).

They are also being explored for their potential use as fungicides in agriculture (He et al., 2010), as anticancer drugs and in biomedical imaging applications (Rasmussen et al., 2010; John et al., 2010). The increased production and use of ZnO-NPs enhances the probability of exposure in occupational and environmental settings. They may be introduced to the environment through wastewater from industrial sites or domestic sewage from showering or swimming. NPs can be transported to soil *via* sewage sludge used for land application. Therefore, terrestrial ecosystems are expected to be an ultimate sink for a larger portion of NPs. This raises concerns about their ecological effects, entry into food webs, and ultimately, human exposure from the consumption of contaminated agricultural products. Hence, it is of great interest to understand the effect of NPs on soil organisms.

In this study, earthworm *Eisenia fetida* was exposed to ZnO-NPs because it occupies 60 to 80% of the total biomass and is well known for its contribution to lingocelluloses' decomposition of organic wastes. Earthworms (EWs) influence decomposition indirectly by affecting microbial population structure and dynamics. The gut of some species of earthworm poses cellulolytic activity (Siturzenbaun, 2009). However, it has long been recognized that most earthworms and other animals living in the soil do not produce their own endogenous cellulase; instead they depend on the cellulase from their resident gut microorganism (Domínguez et al., 2005). However, endogenous cellulase genes have been recently reported from earthworm *Pheretima hilgendor* (Nozaki et al., 2009). Despite these newly discovered abilities, earthworm cannot assimilate lignocellulose by means of endocellulase alone, since efficient lignocellulose degradation requires synergetic action of a suite of other enzymes, including exocellulase, hemicellulase (xylanase) and lignin peroxidase (Lynd et al., 2002). According to Brown and Doube (2004), a synergistic earthworm-microbial digestive system (dual-digestive system) is indispensible for the digestion and utilization of lingocellulose by earthworms. Several studies have demonstrated that earthworm gut contain aerobic microorganism in abundance (Dash et al., 1986; Karsten and Drake, 1995). Moreover, some aerobes have been shown to proliferate during passage through the earthworm gut, reaching densities greater than those in the soil (Fischer et al., 1995; Kristuek et al., 2007; Parthasarathi et al., 2007); considering the dual-digestive system described and the abundance of aerobes in earthworm gut, we hypothesized that some species of cellulolytic aerobes can survive when exposed to ZnO-NPs and may also contribute to lignocelluloses digestion in gut.

Our current understanding of the potential impact of nanoparticles on cellulolytic activity of earthworms is limited. In earlier studies, we reported that the application of 100 nm and 50 nm ZnO-NPs showed no significant DNA damage on *E. fetida* and recorded their coelomocyte potential ability to uptake ZnO-NPs from soil ecosystem (Gupta et al., 2014). The question of the impact of ZnO-NPs on earthworm-gut associated microbiota needs to be answered. The study suggests, ZnO-NPs enhanced the activity of cellulolytic bacteria in the gut of *E. fetida.*

MATERIALS AND METHODS

Test compound

ZnO-NPs (100 nm, 50 nm, 35 nm, 10 nm) were purchased from Sigma Aldrich chemical (St. Louis, MO, USA). Size of particles was measured in 20 µl particle suspension from the test medium on 400 mesh carbon-coated copper grid and was observed in TEM (40-100 kv) at the Sophisticated Analytical Instrumentation Facility, Department of Anatomy, All India Institute of Medical Science, Delhi, India.

Test organism and method of exposure

Exposure of ZnO-NPs on *E. fetida* followed the published organisation for Economic Co-operation and Development (OECD) guideline (2004). Twenty-clitellate adult E. fet*ida* weighing 0.30±0.12 g in three replicate exposure chamber, containing 1 kg dry mass of artificial soil medium were chosen for the test experiment. The soil medium consists of 70% quartz sand, 10% peat moss and 20% kaolin. The pH was adjusted with the addition of a small amount of crushed limestone. Various doses (He et al., 2010) of NPs (0.5-10.0 µg/kg) were added to the dry soil and mixed by homogenizer for 5 min. The moisture content was maintained for 60%. After 40 days of exposure, earthworms were washed with autoclaved tap water; their body surface were sterilized by a brief rinse with 70% ethanol and immediately anesthetized on crushed ice.

Estimation of Zn content in earthworms' tissue

Three EWs of each group were weighed and digested with 3 ml of concentrated nitric acid for 24 h. After digestion, the acid was evaporated; then the residue was dissolved in 4% nitric acid. Zinc concentration was quantified with Inductively coupled plasma atomic emission spectroscopy (ICP-AES) at The Energy Research Institute, New Delhi, India.

Enzymatic activities

Cellulase activity was estimated by determining the released reducing sugars after the incubation of samples (5 g fresh weight) with carboxymethyl cellulase (CMC), sodium salt (0.7%) for 24 h at 50°C in a 690 nm microplate reader (Schinner and Von Mersi, 1990).

Isolation of cellulolytic bacteria

The complete intestine was dissected out and homogenized in autoclaved distilled water, containing 0.5 mm glass beads with

vortex mixing for 5 min. The resulting suspension was serially diluted with water and used as inoculum. Cellulose-hydrolytic bacteria were isolated using Bushnell Hass medium (BHM) amended with carboxymethyl cellulase (CMC) as the sole carbon source. The CMC- amended with BHM medium consist of (g/l); CMC, 10; $MgSo_4.7H_2O$, 0.2; K_2HPo_4, 1; KH_2Po_4, 1; $NH_4NO_3$1; $Fecl_3.6H_20$, 0.05 and $CaCl_2$, 0.02. After enrichment in CMC amended medium, the inoculums (0.1 ml; successively diluted to 10^{-5} times) were repeatedly streaked on BHM agar plates containing the amended CMC. After one week of incubation, the plates were stained by Congo red to observe cellulolytic activity of isolated strains. The cellulase activity of each culture was determined by measuring the zone of clearing on agar plate. The individual colony having significant clear zone was selected and transferred to a fresh CMC-amended BMH medium; then the inoculum was serially diluted 10^{-5} times and streaked over BMH agar plates repeatedly, and the bacteria were re-isolated. Through several such processes, eighteen pure bacterial cultures were obtained, morphologically observed in light microscope and biochemically characterized using Garrity et al. (1985) method.

16S rRNA gene sequencing and phylogenetic analysis of isolates

Amplification and sequence analysis of the 16S rRNA gene was performed as described by Chen et al. (2001). The sequences of isolates were compared with other available sequences in the gene bank. The multiple sequence alignment including the eighteen cellulose-degrading strains and their close relatives were obtained using National Center for Biotechnology Information (NCBI) platform. The phylogenetic reconstruction was inferred using the neighbor-joining method UPGMA, maximum-likelihood and Fitch-Margoliash methods BioEdit software in the Bio Edit program. A phylogenetic tree was drawn using the TREE VIEW program. The sequence identities were calculated using NCBI software.

Nucleotide sequence accession numbers

The 16S rRNA gene sequences of the bacterial isolates have been deposited in NCBI nucleotides sequence databases. Obtained accession numbers of the isolates are shown in Table 1.

Statistical analysis

Two-way analysis of variance (ANOVA) was performed using the Statistical Package for the Social Science (SPSS) 10.5 software. The objective of the statistical analysis was to determine any significant differences among the parameters analyzed in different treatments during the determination of uptake of ZnO-NPs and their potential use as a biotransformation agent.

RESULTS

Survivability of earthworms exposed to ZnO-NPs

The survivability of EWs was observed (Table 2) after exposure of a wide range (0.5 to 10 mg/kg) of ZnO-NPs (100, 50, 35 and 10 nm). It was observed that *E. fetida* could survive even at high concentration (10 mg/kg). Commercially, ≤0.5 mg/kg ZnO-NPs are used for different purposes including nanofertilizer for the release of Zn in soil ecosystem. Thus, the highest concentration was considered to be 10 mg/kg. However, there was sporadic mortality in some treatments, which were neither concentration-dependent nor statistically-significant.

Zn content in EW's tissue

After 28 days of exposure, the Zn content in body tissue was measured (Table 3); the result exhibits increase in bioaccumulation with decrease in size of NPs. The highest Zn content (36.18±3.17 µg/kg) was recorded at the exposure of 10 nm. The results suggest that EWs can uptake and accumulate 20 to 36 µg/g Zn in their body tissue, depending on the dose and size of ZnO-NPs. Gardea-Torresdey et al. (2005,) observed that Au^{+1} and Au^{+3} can also be consumed by EWs from the soil and can subsequently be reduced to metal within the tissues. To our knowledge, this is the first evidence to prove that this process also account for the reduction of ZnO to Zn by EWs.

Increase in cellulolytic activity

The cellulolytic activity of EWs' gut increased with the decrease in size of NPs (Table 4). Although, no significant variations were observed in cellulolytic activity as compared to the control for 100 and 50 nm exposures at ≤7.5 mg/kg. In contrast, the exposure of 35 and 10 nm ≥3.5 mg/kg sized NPs, showed increase in enzymatic activity by 38 to 41%. Increase in cellulolytic activity in EWs may be helpful in bioconversion of lignocelluloses waste. Degradation of cellulose is a slow process limited by several factors involving cellulases (Sinsabaugh and Linkins, 1988). Decomposition of lignocellulosic residues is directly mediated by extracellular enzymes (Sinsabaugh et al., 1992). Therefore, analysis of the dynamics involved in the increase of cellulolytic activity with the exposure of ZnO-NPs may clarify the mechanism relating to the rate of decomposition with substrate quality and nutrient availability as reported by Sinsabaugh and Linkins (1993). Observations are contrary to Hu *et* al. (2010) who reported that NPs adversely affects cellulase activity in *E. fetida.*

Phylogenetic analysis of cellulolytic bacterial isolates

Eighteen strains of cellulose hydrolytic bacteria (Table1), capable of producing cellulase, were obtained from the gut of EWs exposed to ZnO-NPs. All strains grew well at 35°C on CMC-amended BHM medium under aerobic conditions. Colonies on CMC agar plates are circular, smooth, creamy yellow circles within 3 days (Figure 1). Microscopic examination showed that the isolated strains were in straight rods with Gram-positive reaction. The

Table 1. Accession numbers of bacterial isolates obtained by submission of 16S rRNA sequence to NCBI.

Strain	Scientific name	NCBI accession no.
Bs 1	*Bacillus licheniformis*	KC936877
Bs 2	*Bacillus sp.*	KC915013
Bs 4	*Bacillus subtilis*	KC915014
Bs 5	*Bacillus subtilis*	KC936879
Bs 6	*Brevibacillus limnophilus*	KC936880
Bs 8	*Pseudomonas aeruginosa*	KC936881
Bs 9	*Pseudomonas aeruginosa*	KC905036
Bs 10	*Pseudomonas aeruginosa*	KC936078
Bs 11	*Bacillus sp.*	KC953043
Bs 12	*Paenibacillus alvei*	KC894745
Bs 13	*Paenibacillus sp.*	KC953038
Bs 14	*Paenibacillus alvei*	KC953041
Bs 19	*Bacillus sp.*	KC953040
Bs 20	*Pseudomonas aerugi*	KC953036
Bs 21	*Enterobactor sp.*	KC953037
Bs 25	*Pseudomonas aeruginosa*	KC953039
PR 7	*Paenibacillus dendritiformis*	KC953042
PC 7	*Paenibacillus dendritiformis*	KC905037

Table 2. Percentage of mortality of *E. fetida* in ZnO-NPs fortified vermireactors.

Dose (mg/kg)	100 nm exposure	50 nm exposure	35 nm exposure	10 nm exposure
0	5±0.84	6±0.60	5±1.14	6±0.89
0.5	5±0.83	5±0.89	6±1.24	6±1.04
1.0	6±0.54	5±0.83	5±1.20*	8±1.24*
1.5	8±0.89	6±0.70*	6±2.10	6±2.34
2.0	6±1.23*	6±1.14	6±3.40	6±2.48*
2.5	6±0.78	6±0.89	8±1.10	6±2.14
3.0	5±0.00	6±1.05	6±2.18	5±1.60
3.5	6±0.44	7±0.83	6±0.34*	6±1.44*
4.0	8±1.03	6±0.89*	6±0.88	7±1.44
4.5	6±0.70*	6±0.86	8±1.70	6±2.15*
5.0	8±0.80	6±1.60	7±1.80	7±2.44
5.5	7±1.14	6±1.90*	8±1.90	10±2.14
6.0	8±1.09	8±0.88	8±2.14*	10±0.88
6.5	8±0.89*	9±2.36	8±2.30	11±1.74*
7.0	7±0.54	8±1.70	8±1.20	12±1.88
7.5	6±0.83	8±1.85	6±1.20	18±2.14
8.0	8±0.80*	6±2.14	8±1.30	20±1.14
8.5	7±0.70	8±1.40*	8±1.30*	28±1.68*
9.0	8±1.45	7±1.40	7±2.14	26±1.88
10.0	9±1.60	8±3.14	6±2.14	28±1.74

All values are mean and standard deviation of three replicates.*Significant ($p<.01$).

16S rRNA gene were also used to characterize cellulolytic isolates (Figure 1). Based on the sequence identity of 16S rRNA gene strains, bs4 and bs5 showed the highest similarity towards the *Bacillus subtilis* type strain. Strain b1 exhibits close resemblance (99.8%) with *Bacillus licheniformis*. Strains bs2, bs 11 and bs19 represent different sublines of *Bacillus.*

Moreover, bs8, bs9, bs10, bs20 and bs25 showed

Table 3. Zinc accumulation (µg/g) in body tissue of *E. fetida* after (28 days) exposure of ZnO NPs.

Dose (mg/kg)	100 nm exposure	50 nm exposure	35 nm exposure	10 nm exposure
0	4.04±0.20	4.08±0.45	4.00±0.45	4.00±1.14
0.5	4.13±0.23*	4.12±0.78	4.56±0.60	5.58±0.70*
1.0	4.36±0.34*	4.56±0.66	4.80±0.60	7.24±0.68*
1.5	4.46±0.33*	4.58±0.33*	5.44±0.50*	7.80±2.10*
2.0	4.78±0.45	4.82±0.78*	5.65±0.38*	7.84±0.80
2.5	4.88±0.84*	4.90±0.23	5.70±0.70*	8.90±0.84
3.0	5.80±0.64	7.34±0.89*	7.84±0.80	10.10±0.90*
3.5	7.28±1.14	8.14±0.23	10.32±0.64*	16.14±1.14
4.0	7.56±0.74*	9.98±0.56*	12.45±2.14	18.86±1.18
4.5	7.46±0.74*	10.96±0.56	14.50±2.64	20.47±2.14
5.0	9.48±0.74	12.06±1.10	18.98±3.10	24.37±2.78*
5.5	10.45±0.84	13.26±0.78*	18.64±1.14*	28.40±2.14*
6.0	10.45±1.09	14.80±0.64*	19.40±0.20	27.56±2.24
6.5	14.67±1.09	16.80±0.74*	22.34±2.14*	32.16±4.12*
7.0	17.45±0.78*	18.78±0.56	24.67±3.20	35.16±3.12
7.5	18.45±0.64*	18.34±0.65	23.46±1.10*	34.17±4.14*
8.0	18.30±0.84*	18.74±2.45*	22.56±2.18*	36.18±3.17*
8.5	18.24±2.14*	18.78±0.80	22.64±2.10	36.12±2.14
9.0	18.14±0.94*	18.80±1.10	23.34±2.10	36.12±4.98*
10.0	18.14±1.44	18.80±0.84*	23.45±1.14	36.12±5.14

All values are mean and standard deviation of three replicates.*Significant (p<.01)

Table 4. Cellulolytic Activity (µg/g/h) in gut of *E. fetida* after the exposure of various ZnO-NPs.

Dose (mg/kg)	100 nm	50 nm	35 nm	10 nm
0	176.0±0.96*	175.0±6.56	176.3±7.08	174.5±8.44
0.5	179.2±2.52	173.6±9.14*	164.0±7.24*	176.5±7.77*
1.0	173.4±1.68*	176.9±2.04	189.1±5.04*	176.0±9.62*
1.5	174.4±0.47	176.3±0.71*	173.6±2.28*	196.7±0.82
2.0	174.6±5.32	176.9±2.06	171.1±3.49	203.1±6.68*
2.5	174.3±0.98*	176.7±1.46	168.3±3.22	196.4±3.36
3.0	183.2±416*	181.1±1.16*	152.7±3.58	220.3±4.34
3.5	177.8±8.24	178.2±9.46	167.9±8.88*	237.3±8.28
4.0	176.5±7.84*	179.2±6.28	170.7±3.72*	234.9±7.78*
4.5	187.9±1.38*	173.7±5.16	170.8±0.58	236.3±7.06
5.0	172.5±6.92	174.2±4.13	-	248.0±3.26*
5.5	168.6±5.07	181.4±5.54*	165.1±3.03	255.5±9.02
6.0	183.5±1.66	175.8±2.12	174.3±3.98*	236.3±4.52
6.5	180.6±3.08*	173.0±4.44	171.9±2.05*	242.4±6.72
7.0	173.7±6.02	-	177.2±2.64	242.3±2.96*
7.5	177.9±9.48*	183.4±1.66	166.4±7.72	246.3±1.46*
8.0	184.8±3.45	182.7±9.82*	163.8±6.64	239.0±3.18*
8.5	186.2±7.74*	181.5±3.02	-	251.8±1.12
9.0	187.1±7.06	185.8±9.62	240.7±4.94	245.3±4.53
10.0	185.8±3.38*	181.5±6.16	243.1±2.02*	249.1±3.18

All values are mean and standard deviation of three replicates.*Significant (p<.01)

close similarity (99.8%) with type strain *Pseudomonas aeruginosa.* The highest similarity of bs12 and bs14 was found close to *Paenibacillus alvei*. PR7 and PC7 show 99.9% similarity with *Paenibacillus dendritiformis*. Strain bs13 represents different sublines of *Paenibacillus* and bs21 belongs to *Enterobacter.* Strain bs6 shows close similarity (99.9%) with *Brevibacillus limnophilus;* and is reported for the first time in EWs'gut to the best of our

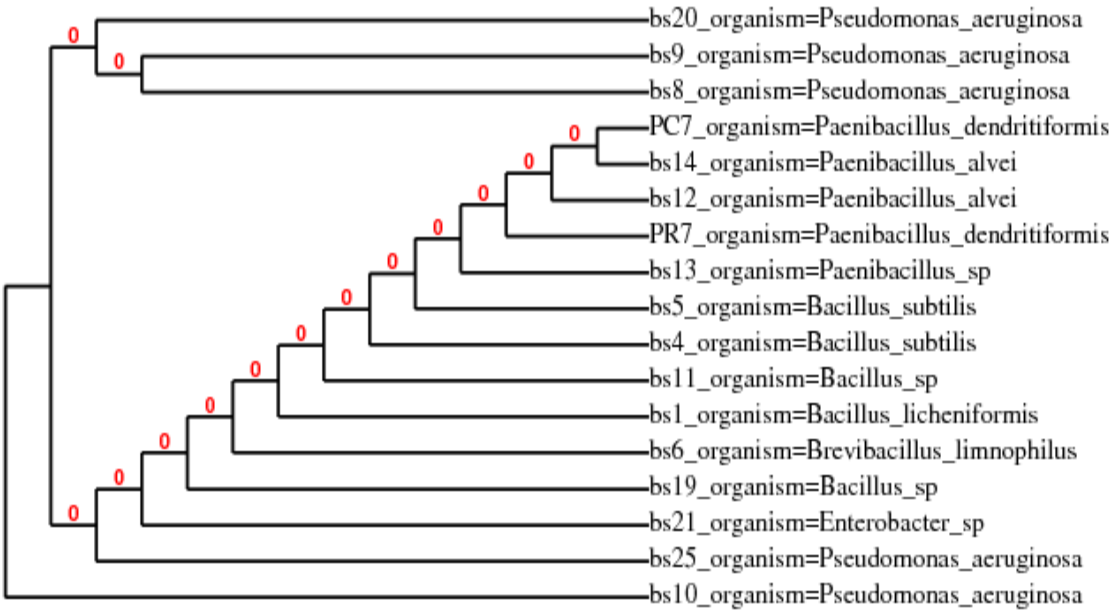

Figure 1. Phylogenetic tree of cellulose hydrolytic isolates of gut of *E. fetida* constructed using the Robust Phylogenetic Analysis software (LIRMM).Trees for bacterial isolates are based on the partial 16r DNA sequences.

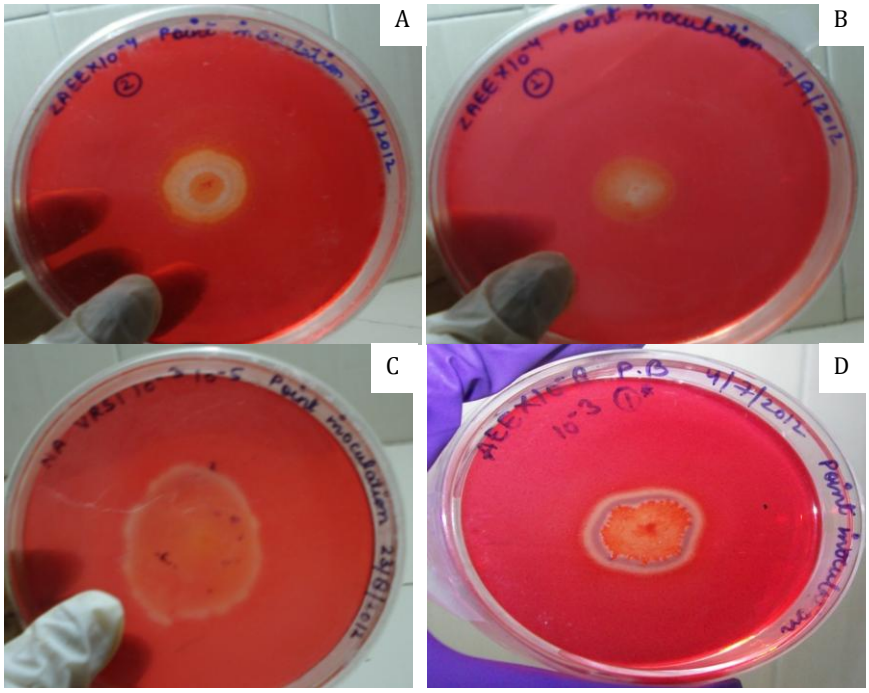

Figure 2. CMC test of *B. licheniformis* (A); *B. subtilis (B); P. aeruginosa* (C); *P. dendritiformis* (D) isolated from EWs' gut exposed to ZnO –NPs spiked reactors.

knowledge. The bio-chemical properties of isolated cellulose hydrolytic bacteria of EWs' gut exposed to ZnO-NPs are shown in Table 5. The 16S rRNA gene phylo-genetic tree using the neighbor-joining method was constructed for the eighteen strains as indicated in Table 1. According to the results of the biochemical and the 16SrRNA gene sequence examinations, the six strains (bs1, bs2, bs4, bs5, bs11 and bs19) belongs to *Bacillus* sp.; another

Table 5. Biochemical properties of eighteen bacterial isolates from EWs' gut exposed to ZnO –NPs fortified vermireactor.

Strain	Isolate	Gram staining	Motility	Aerobic	Shape	Citrate utilization	Gelatin liquification	Catalase positive
bs1	*Bacillus licheniformis*	+	+	+	Rod-spore forming	-	+	+
bs2	*Bacillus sp.*	+	+	+	Rod-spore forming	+	+	+
bs4	*Bacillus subtilis*	+	+	+	Rod-spore forming	-	+	+
bs5	*Bacillus subtilis*	+	+	+	Rod-spore forming	-	+	+
bs6	*Brevibacillus limnophilus*	+	+	+	Rod-spore forming	+	+	+
bs8	*Pseudomonas aeruginosa*	-	+	+	Rod-non spore	+	+	+
bs9	*Pseudomonas aeruginosa*	-	+	+	Rod-non spore	+	+	+
bs10	*Pseudomonas aeruginosa*	-	+	+	Rod-non spore	+	Late	+
bs11	*Bacillus sp.*	+	+	+	Rod-sporeforming	+	+	+
bs12	*Paenibacillus alvei*	+	+	+	Rod-spore forming	-	+	+
bs13	*Paenibacillus sp.*	+	+	+	Rod-spore forming	+	+	+
bs14	*Paenibacillus alvei*	+	+	+	Rod-spore forming	-	+	+
bs19	*Bacillus sp.*	+	+	+	Rod-spore	-	+	+
bs20	*Pseudomonas aeruginosa*	-	+	+	Rod-non spore	+	+	+
bs21	*Enterobacter sp.*	-	+	+	Rod-non spore	-	late	+
bs25	*Pseudomonas aeruginosa*	-	+	+	Rod-non spore	+	+	+
PR7	*Paenibacillus dendritiformis*	+	+	+	Rod-sporeforming	+	+	+
PC7	*Paenibacillus dendritiformis*	+	+	+	Rod- spore forming	+	+	+

Strain	Isolates	H2S production	Nitrate reduction	Starch hydrolysis	Glucose	Sucrose	Mannitol	Methyl red	Voges proskauer
bs1	*Bacillus licheniformis*	+	-	+	+	+	+	-	+
bs2	*Bacillus sp.*	-	+	+	+	+	+	+	-
bs4	*Bacillus subtilis*	+	+	+	+	+	+	+	+
bs5	*Bacillus subtilis*	+	weak	+	+	+	+	+	+
bs6	*Brevibacillus limnophilus*	-	+	+	+	+	+	+	+
bs8	*Pseudomonas aeruginosa*	-	+	+	+	+	+	-	-
bs9	*Pseudomonas aeruginosa*	-	+	+	+	+	weak	-	-
bs10	*Pseudomonas aeruginosa*	-	+	+	+	+	+	-	-
bs11	*Bacillus sp.*	-	+	+	+	+	-	+	weak
bs12	*Paenibacillus alvei*	+	+	-	+	+	+	-	-
bs13	*Paenibacillus sp.*	-	+	weak	+	+	+	-	-
bs14	*Paenibacillus alvei*	+	+	-	+	+	+	-	weak
bs19	*Bacillus sp.*	-	-	+	+	-	-	-	-
bs20	*Pseudomonas aeruginosa*	-	+	+	+	+	weak	-	-
bs21	*Enterobacter sp.*	-	-	-	+	+	+	-	-
bs25	*Pseudomonas aeruginosa*	-	+	+	+	+	+	-	-
PR7	*Paenibacillus dendritiformis*	+	+	+	+	+		-	-
PC7	*Paenibacillus dendritiformis*	+	+	+	+	+	+	-	-

five strains (bs12, bs13, bs14, PR7 and PC7) belongs to sublines of *Bacillus* under genus *Paenibacillus*; single strain b6 belongs to *Brevibacillus sp.* Furthermore, *another* five strains (bs8, bs9, bs10, bs20 and bs25) belong to *Pseudomonas* sp. Single strain (bs21) of *Enterobacter* sp. was also reported in the gut extract. In the present study, *Bacillus* sp was do-minated in the cellulolytic bacterial biota of EWs' gut followed by *Paenibacillus and Pseudomonas.*

DISCUSSION

To degrade lignocelluloses in ZnO-NPs exposed earthworms, the presence of cellulolytic aerobes in the earthworm's gut is significant. Since, the gut is free of oxygen and anaerobic bacteria, which comprise of a major population in the gut micro-biota (Karsten and Drake, 1995; Wust et al., 2011). Aerobic soil micro-organisms, including cellulolytic ones, associated with lignocelluloses are ingested by an earthworm and then introduced into the anterior digestive tract, where the zinc rich oxic conditions allow their growth and produce enzymes for lignocelluloses digestion. Subsequently, the microorganisms are exposed to the anoxic conditions of the gizzard and intestine, resulting to the activation of aerobic microorganisms with continuous activity of their enzymes, including those of saccharification lingo-celluloses during gut passage. The resulting degradation products (glucose, xylose and their oligosaccharides) are thus consumed by the earthworm and, also used by anaerobes as a fermentation substrate (Fujii et al., 2012). The grown biomass of anaerobes is digested and consumed by earthworms as a source of essential amino acids and fatty acids (Sampedro et al., 2006).

Glycosidase activity should remain even if the growth of enzyme-producing microorganisms is halted by anaerobiosis, because these enzymes are resistant to proteolytic inactivation and are able to continue the saccrification for several hours, which corresponds to the food transit time of earthworms (Dash et al., 1986; Lynd et al., 2002). The growth of cellulose hydrolysis bacteria as well as other aerobes is halted by anaerobiosis, cas-ting these microorganisms to become a minor population in the gut. However, some are resistant to digestion and finally excreted as a part of the cast, because earthworm digestive fluid contains microbial activity that is selective for certain species, but neutral or stimulating for the growth of others (Byzov et al., 2007; Khomyakov et al., 2007).

ZnO nanoparticles have a broad spectrum of antibacterial activities. An inhibitory effect of ZnO nanoparticles on *B. subtilis*, *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pyogenes* and *Enterococcus faecalis* has been reported (Xie et al., 2011; Vani et al., 2011; Wang et al., 2012). Contrary to the earlier findings, our results showed that ZnO nanoparticles favors the bacterial activity in EWs'gut. In the case of the behavior of NPs particles in EWs, two central questions arise: Do particles remain in their nanoparticulate state in gut of EWs or do they congregate to coarse agglomerates/ aggregates?; Do substances such as humic acid that exist in the Ews'gut influence agglomeration behavior?

In this study, we assumed that the particle looses their nanoparticulate state and agglomerate in the gut of EWs due to the presence of humic acid. The release of engineered nanoparticles into the soil ecosystem leads to the contact of these particles with microbiota of EWs'gut; this contact does not affect EWs'microbiota.

The growth of microbiota due to nanoparticle-specific characteristics of metal oxide nanoparticles depends on the dispersion and stabilization of these particles. Our earlier findings, suggests that ZnO particles do not generated significant hydrogen peroxide in the gut of EW (Gupta et al., 2014). This indicates that ZnO-NPs do not remain in the nanoparticulate state of EWs'gut; it aggregates in the gut of the EWs and confirms their biotransformation ability. Therefore, microbiota of EWs' gut is not adversely affected by nanoparticles.

Conclusion

ZnO-NPs are among the most commonly utilized group of nanomaterials and have a wide range of application. As a well-known phytocatalyst, ZnO-NPs have received much attention in the degradation and complete mineralization of environmental pollutants. Finding suggests that the cellulase activity in earthworm gut increased with the decrease in size of ZnO-NPs. Therefore, the analysis of the dynamics involved in the increase of cellulolytic activity with exposure of ZnO-NPs may clarify the mechanisms relating to the rate of decomposition with substrate quality and nutrient availability. The isolated and identified eighteen efficient cellulolytic bacteria have the ability to hydrolyze different cellulosic substrates in ZnO-NPs spiked vermireactors. However, further investigations are required to understand the mechanism that proliferate growth of cellulose-hydrolytic bacteria by ZnO-NPs.

ACKNOWLEDGEMENT

We acknowledge the financial support of the Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi for the support given to us to carry out this study.

Conflicts of interest

The authors declare that they have no conflicts of interest.

REFERENCES

Brown GB, Doube BM (2004). On earthworms assisted bioremediation. In: Edward CA (Ed.), Earthworm Ecology. CRC Press, Boca Raton, Fl, pp. 213-239.

Byzov BA, Khomyakov NV, Kharin SA , Kurakov AV (2007). Fate of soil bacteria and fungi in the gut of earthworms. Eur. J. Soil Biol. 43:146-156.

Chen WM, Tseng ZJ, Lee KS, Chang JS (2005). Fermentative hydrogen production with *Clostridium butyricum* CGSS isolated from anaerobic sewage sludge. Int. J. Hydrogen Energy 30:1063-1070.

Dash HK, Beura BN, Dash MC (1986). Gut load, transit time gut micro flora and turnover of soil plant and fungal material by some tropical earthworms. Pedobiologia 29:13-20.

Domínguez J, Ferreiro A, Velando A (2005). Are *E.fetida* (Savigny, 1826) and *Eisenia andrei* Bouché, 1972 (Oligochaeta, Lumbricidae) different biological species? Pedobiologia 49:81-87.

Fischer K, Hahn D, Amann RI, Daniel O, Zeyer J (1995). *In situ* analysis of the bacterial community in the gut of the earthworm *Lumbricus terrestris* L. by whole-cell hybridization. Can. J. Mirobiol. 41:666–673.

Fujii K, Ikeda K,YoshidaS (2012). Isolation and characterization of aerobic microorganisms with cellulolytic activity in the gut of endogeic earthworms. Int. Microbiol. 15:121-130.

Gardea - Torresdey JL, Rodrigues E, Parsons JG, Peraetavidea JR, Meitzner G, Cruz - Jinenez G (2005). Use of ICP & XAS to determine the enhancement of gold phyto-extraction of *Chilopsis linearis* using thiocyonate as a complexing agent. Anal. Biomal. 382: 347-352.

Garrity GB, David R, Castenholz RW (1985). Bergey's Manual of Systematic Bacteriology. 2nd Edition, Volume 1. Springer, New York, USA.

Gerloff K, Albrecht C, Boots AW, Forster L, Schins RPF (2009). Cytotoxicity and oxidative DNA damage by nanoparticles in human intestinal caco-2 cells. Nanotoxicology 3(4):355-364

Gupta S, Kushwah T, Yadav S (2014). Earthworm coelomocytes as nanoscavenger of ZnO NPs (2014). Earthworm Coelomocytes as nano-scavanger for ZnO NPs. Nanoscale Res. Lett. 9:259.

He L, Liu Y, Mustapha A, Lin M (2010). Antifungal activity of zinc oxide nanoparticles against *Botrytis cinerea* and *Pencillium expansum*. Microbiol. Res.

Hu CW, Li M, Cui YB, Li DS, Chen J, Yang LY (2010). Toxicological effects of Tio_2 and ZnO nanoparticles in soil on earthworm *E. fetida*. Soil Biol. Biochem. 42:586-591.

Jin T, Sun D, Su JY, Zhang H, Sue HJ (2009). Antimicrobial efficacy of zinc oxide quantum dots against *Listeria monocytogens*, *Salmonella enteritidis* and *Escherichia coli* 0157: H7. J. Food Sci. 74(1):46-52.

John S, Marpu S, Omary Li J, Fujita Y (2010). Hybrid zinc oxide nanoparticles for biophotonics. J. Nanosci. Nanotechnol. 10(3):1707-1712.

Karsten GR, Drake HL (1995).Comparative Assessment of the Aerobic and Anaerobic Micro floras of Earthworm Guts and Forest Soils. Appl. Environ Microbiol. 61(3):1039-1044.

Khomyakov NV, Kharin SA, Nechitailo TYu, Golyshin PN, Kurakov AV, Byzov BA, Zvyagintsev DG (2007). Reaction of microorganisms to the digestive fluid of earthworms. Microbiology 76:45-54.

Lynd LR, Weimer PJ, Van Zyl WH, Pretorious IS (2002). Microbial cellulose utilization: Fundamentals and Biotechnology. Microbiol. Mol. Biol. Rev. 66:506-577.

Nel A, Xia T, Madler L (2006). Toxic potential of materials at the nanolevel. Science 2:311:622-627.

Nozaki M, Miura C, Tozawa Y, Miura T (2009). The contribution of endogenous cellulose to the cellulose digestion in the gut of earthworm (*Pheretima hilgendorfi*: Megascolecidae). Soil Biol. Biochem. 41:762-769.

Parthasarathi K, Ranganathan LS, Anandi V, Zeyer J (2007). Diversity of microflora in the gut and casts of tropical composting earthworms reared on different substrates. J. Environ. Biol. 28:87-97.

Rasmussen JW, Martinez E, Louka P, Wingett DG (2010). Zinc oxide nanoparticles for selective destruction of tumour cells and potential for drug delivery applications. Expert Opin. Drug Deliv. 7(9):1063-1067.

Sampedro L, Jeannotte R, Whalen JK (2006).Trophic transfer of fatty acids from gut microbiota to the earthworm *Lumbricus terrestris*. Soil Biol. Biochem. 38:2188-2198.

Schilling K, Bradford B, Castelli D, Dufour E, Nash JF, Pape W (2010). Human safety review of "nano" titanium dioxide and zinc oxide. Photochem. Photobiol. Sci. 9(4):495–509.

Schinner F, Von Mersi W (1990). Xylanase-CM-cellulase- and invertase activity in Soil: an improved method. Soil Biol. Biochem. 22: 511–515.

Sinsabaugh RL, Antibus RK, Linkins AK, Mcclaugherty CA, Rayburn L (1992). Wood decomposition over a first- order watershed: mass loss as a function of lignocellulase activity. Soil Biol. Biochem. 24:743-749

Sinsabaugh RL, Linkins AE (1993). Statistical modeling of litter decomposing from integrated cellulase activity. Ecology 74:594-1597.

Sinsabaugh RL, Linkins AE (1988). Adosorption of cellulose components by leaf litter. Soil Biol. Biochem. 20:927-931.

Sturzenbaum S (2009). Earthworm and nematode metallothioneins in metal ions in life sciences. Royal Soc. Chem. 5:183-197

Vani C, Sergin GK, Annamalai A (2011). A study on the effect of zinc oxide nanoparticles in *Staphylococcus aureus*. Int. J. Pharm. Biol. Sci. 2(4):324-327.

Wang C, Liu LL, Zhang AT, Xie, P, Lu JJ, Zou XT (2012). Antibacterial effects of zinc oxide nanoparticles on *Escherichia coli* K88. Afr. J. Biotechnol. 11(44):10248-10254.

Wust PK, Horn MA, Dake HI (2011). *Clostridiaceae* and *Enterobacteriaceae* as active fermenters in earthworm gut content. ISME 5:92-106.

Yanping, He Yiping, Peter L Irwin, Tony Jin, Xianming Shi (2011). Antibacterial Activity and Mechanism of Action of Zinc Oxide Nanoparticles against *Campylobacter jejuni.* Appl. Environ. Microbiol. 77(7):2325-2331.

academicJournals

Vol. 7(2), pp. 18-23, February 2015
DOI: 10.5897/JTEHS2015.0327
Article Number: 32A440A50527
ISSN 2006-9820
Copyright © 2015
Author(s) retain the copyright of this article
http://www.academicjournals.org/JTEHS

Journal of Toxicology and Environmental Health Sciences

Full Length Research Paper

Haemato-pathological effect of dichlorvos on blood picture and liver cells of albino rats

Brown Holy[1*], Kenanagha, B[2] and Onwuli, D.O[1]

[1]Department of Medical Laboratory Science, Rivers State University of Science and Technology, Npkolu, Rivers State, Port Harcourt, Nigeria.
[2]Medical Division, Total Upstream Companies Nigeria, Plot 25 Trans Amadi Industrial Layout, Port Harcourt, Rivers State, Nigeria.

Received 28 December, 2014; Accepted 4 February, 2015

The effect of intraperitonal exposure of dichlorvos an organophosphate (OP) pesticide, on haematology parameters and liver pathology of Wister rats was investigated. Thirty male albino rats grouped into six (6) of five rats each were injected with 0, 3.7, 7.4, 11.1, 14.8 and 18.5mg/kg of dichlorvos (DDVP) (1 ml/kg) respectively. The haematological parameters measured were red blood cells, haemoglobin; packed cell volume, total white blood cells count and platelet levels. Histological examination of liver tissue was investigated as well. The result of the haematological parameters of the dichlorvos treated rats showed a significant decrease ($p<0.05$) in the mean values of red blood cells, haemoglobin and packed cell volume and a significant increase ($p<0.05$) in the total white blood cell count and platelet count which was dose dependent. Changes observed in the liver architecture of the treated rat tissues were feathery looks, fatty changes and centrilobular necrosis. However there was no architectural distortion observed in the liver tissue of the control rats. Dichlorvos had dose dependent target toxicity.

Key words: Liver, toxicity, dichlorvos, pestiscides, haematology.

INTRODUCTION

Organophosphates (OPs) today have become the most implicated pesticide in cases of poisoning, they are frequently used as households, garden and farmland insecticides. The importance of pesticide use in Nigeria can be understood from the fact that agriculture is a major component of the Nigerian economy. It contributes 22% of the nation's gross domestic product (GDP) and is the livelihood of nearly 60% of the country's workforce. The widespread use of pesticides in agricultural practice, public health, commerce and individual households results in acute intoxication each year. The first global estimates of the extent of pesticide poisoning were published in 1990 by the World Health Organization (WHO).

WHO estimates based on 2001 data reported that, 849,000 people die globally from self harm each year (WHO, 2002). OPs are highly toxic chemicals that kill insect and other pest by inhibiting the action of the enzyme acetylcholinesterase that functions normally by degrading acetylcholine in nerve synapses. Exposure to OP pesticides had been found to produce adverse effects in exposed populations including humans; such exposures can result in ill health and even death (Michael

*Corresponding author. E-mail: hbinternational2002@yahoo.com. Tel: +234-8038703710.
Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

et al., 2008). The populace is inevitably exposed to OP pesticide poisoning through environmental contamination or occupational use. Occupational exposure can occur at all stages of pesticide formulation, manufacture and application.

The potential adverse impact of dichlorvos on human health is likely to be higher in developing countries like Nigeria, due to easy availability of this highly hazardous chemical product and its low risk awareness among the populace especially local farmers. Individuals can be exposed to dichlorvos through both direct and indirect routes, direct exposure occurs in farmers and individuals who personally apply pesticides in agricultural, occupational or residential settings. Indirect exposure occurs through drinking water, air, dust and foods. This represents routes of long-term low level exposures (Alavanja et al., 2004). As with other organophosphates; dichlorvos is readily absorbed through the skin, as well as by inhalation and from the gastrointestinal tract (Alavanger et al., 2004; Yurumez et al., 2007), acute illness through dichlorvos exposure is limited to the effects of cholinesterase inhibition compared to poisoning by other organophosphates.

Therefore, this present study was aimed at investigating the effects of acute dichlorvos exposure on haematology parameters and liver pathology.

MATERIALS AND METHODS

Test animals

A total of thirty (30) Wister albino rats (*Ratus ratus*) of 150 to 200 g average weight were used for the study. The animals were housed in polypropylene cages under hygienic conditions and were acclimatized for three weeks prior to commencement of the study. The rats were maintained on standard laboratory feed and water *ad libitum* and treated in accordance with the standard guide for the care and use of laboratory animals (NRC, 1985). There was no ethical issue; however it is relevant that the standard guide for the use of laboratory animal be followed.

Animal treatment schedule

Rats were randomly divided into two groups: a control group (n=5) and an experimental group (n=25). The experimental group rats were further divided into five subgroups, each group representing the different dose levels of dichlorvos. The treatments were administered in the morning (8.00 am to 9.00 am) to non fasted rats. The rats were anesthetized with chloform and sacrificed within 2 h of chemical administration.

Acute toxicity study

The acute toxicity study (LD_{50}) entails administering a single dose of the chemical substance (dichlorvos) intraperitoneally into the experimental rats and observing its effects over a short period, usually over 24 h (Dede et al., 1991). The effects of dichlorvos on the tested animals was observed, blood samples were taken for haematological tests. The animals were sacrificed, the liver excised and subjected to histological examination.

Haematological investigation

A general anesthesia was achieved by inhalation of isofluorene. The rat was placed back on a dissecting board, xyphoid process was palpated and 21 G needle was inserted into the ventricle, the plunger was pulled backward slowly to allow blood sample collection into EDTA bottle for haematological investigation. The haematological parameters investigated were red blood cell (RBC), haemoglobin (HB), packed cell volume (PCV), total white blood cell (WBC) and platelet count. All samples were analyzed using ERMA INC. full automatic blood cell analyzer, model PCE- 210N.

Histopathological examination

The Wister (albino) rats were sacrificed and the liver resected. The resected livers were fixed by placing it in plastic jars containing 10% formal aldehyde labelled accordingly. After fixation, the liver tissues were dehydrated by using increasing strength of alcohol: 70, 85, 90 and 100% in an incubation period of 1 h respectively. The tissues were cleared with xylene for 30 min, and were embedded in a mould containing liquid paraffin wax and allowed to solidify. The prepared and embedded tissue block was first trimmed and then secured to a holder which is then mounted on the microtone. The thickness of the sections was reset at 5 mm thickness and 8 to 10 sections of each liver tissue were made. The microscopic slides were labelled using diamond pen accordingly. The thin sections were carefully transferred to water at 45°C in water bath. The labelled microscopic slide were dipped into adhesive solution and slowly pulled upward, out of the solution, allowing sections to adhere to the surface. The bottom of the slide were dried and carefully blotted of excess adhesive from around the sections. The sections were allowed to dry overnight in the storage box. The sections were cleared by passing the mounted sections through the cleaning agent xylene, leaving only the tissue adhering to the slide. The sections fixed on slide were stained with Haematoxylin for 5 min and washed in running water for 30 s. Excess stain were washed in 1% acid alcohol by continuous agitation for 15 s and later washed in running tap water for 30 s. The slides were dipped into ammonia water 2 to 3 times and then washed in running tap water for 30 s. Eosin was applied for 3 to 5 min and then washed in running water for 30 s. These were dehydrated by dipping the slides in increasing concentration of alcohol of 50, 70, 95 and 100% for 2 to 3 min. Then cleared with xylene and mounted with Canada balsam. The stained sections were observed under a light microscope and photographs of the various observations were taken and saved in JPEG format as x400 magnification.

Statistical analysis

Statistical analysis was carried out on the haematological data obtained using Microsoft Excel statistical tools (2003 version). The mean, standard deviation and standard error of mean were calculated. The values were presented as means ± standard error of mean (SEM) and compared by student's statistical test. $P<0.05$ was accepted as statistically significant level.

RESULTS

Male albino rats treated with different doses of dichlorvos (3.7, 7.4, 11.1, 14.8 and 18.5 mg/kg), showed sluggish behaviour, restlessness, micturition, respiratory distress, convulsion and death especially at higher doses of the chemical. These symptoms are typical signs of toxicosis which

Table 1. Toxic Acute Effect of Dichlorvos on Hematological Parameters (mean±SEM)

Group	1	2	3	4	5	6
Does mg/kg	0 (Control)	3.7	7.4	11.1	14.8	18.5
RBC $X10^{12}$/i	7.81.0	7.2±1.2 ^{ns}	6.9±0.5 ^{ns}	5.0±0.8^{a}	4.32±1.2^{a}	3.78±1.0^{a}
HB g/dl	17.5±0.5	15.7±0.9 ^{ns}	14.7±1.0^{ns}	12.8±0.6^{a}	12.0±0.8^{a}	11.5±1.0^{a}
PCV %	52±0.6	47±0.5^{ns}	45±0.8^{ns}	38 ±0.5^{a}	36±0.9^{a}	33±0.9^{a}
WBC $X10^{9}$/i	9.0±1.0	9.7±0.6 ^{ns}	10.5±1.0^{ns}	13.2 ±1.5^{a}	15.5±1.2^{a}	17.0 ±1.5^{a}
PLT $X10^{9}$/i	40±1.0	42±0.6^{ns}	48±1.0^{ns}	53±1.6^{a}	17.0±1.5^{a}	65±1.5^{a}

n=5; ^{a}P<0.05 = significant; ns – not significant; SEM = standard error of mean; n- no of rats.

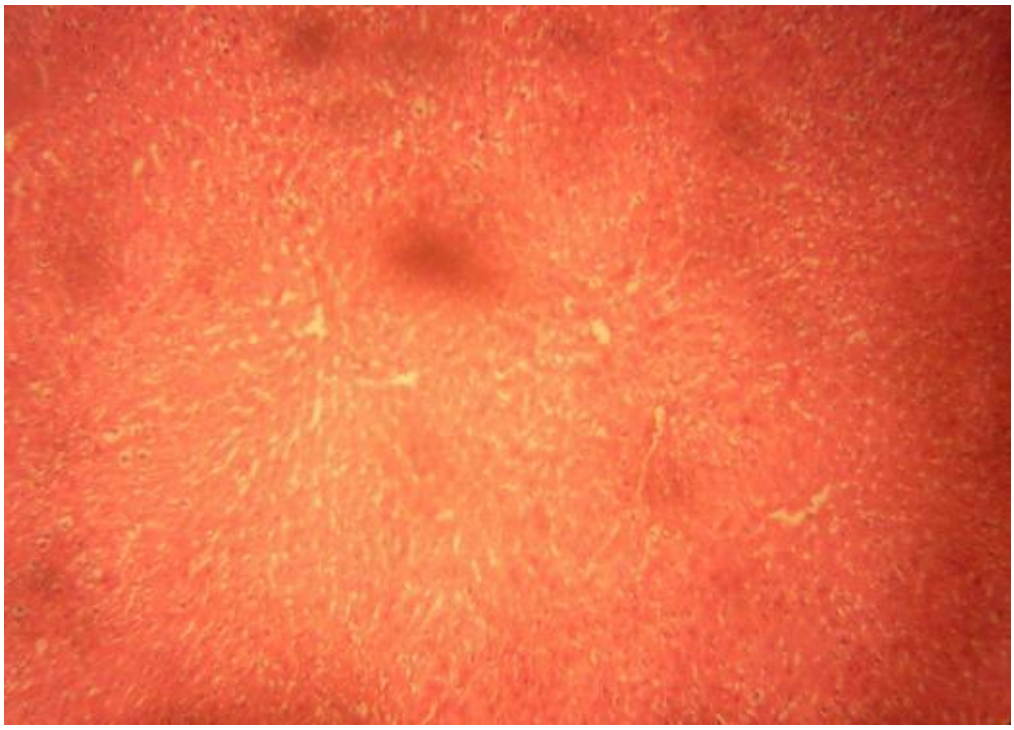

Figure 1. Liver histology of control Wister albino rat (0.9% saline). ×200 magnification. Observation: Normal liver architecture.

which could be attributed to inhibitory action of dichlorvos on the enzyme cholinesterase there, resulting to accumulation of acetylcholine at synapses.

Haematological observations

Table 1 showed haematological indices RBC, HB, PCV, Total WBC and Platelet levels obtained in albino rats exposed to acute dichlorvos poisoning. Statistically dose dependent decreases were observed in the RBC, HB and PCV values obtained ($p< 0.05$) for the exposed albino rats when compared to the control rats. While statistically dose dependent increases were observed in the WBC and platelets values ($p < 0.05$) when compared with the control value.

Histopathological observations

Pathological changes observed in architecture of the liver of rats exposed to acute dichlorvos poisoning that was dose dependent mostly from dose level 11.1 to 18.5 mg/kg. The liver of the dichlorvos treated rats (Figure 1 to 4.) showed morphological changes in the mitochondrial, areas of feathery degeneration, and steatosis. The control rat group showed normal liver architecture.

DISCUSSION

Acute exposure to organophosphate pesticides is often accompanied with serious health hazards. It is established that many of these pesticides can produce some

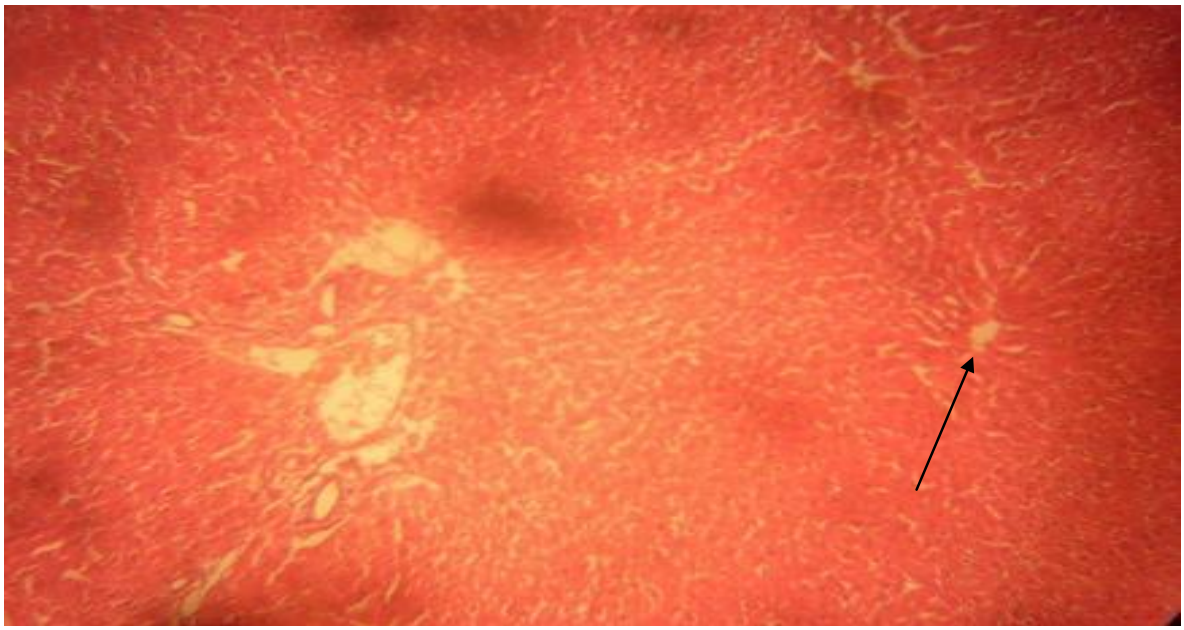

Figure 2. Liver histology of Wister albino rat (7.4 mg/kg dichlorvos). ×200 magnification
Observation: Slight feathery change.

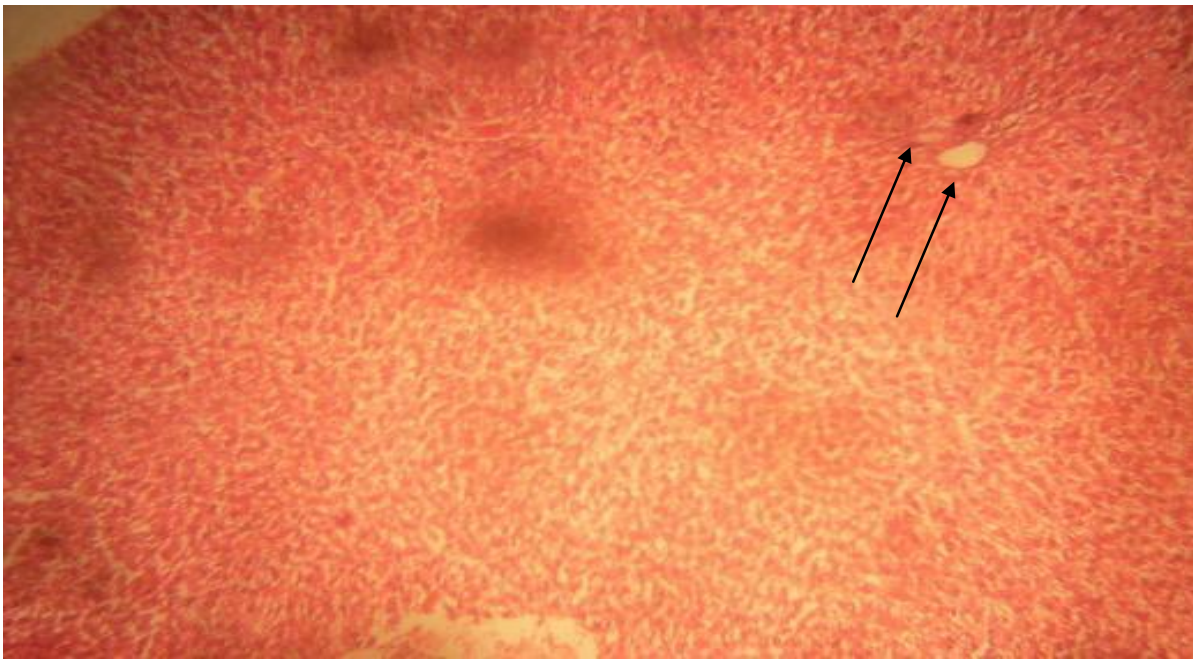

Figure 3. Liver histopathology of Wister albino rat (11.1 mg/kg dichlorvos). ×200 magnification
Observation: Mild feathery change. It represents feathery texture that is observed all over the picture.

adverse effects on the liver and other vital organs in the body through their mode of action or by the production of free radicals (Khan et al., 2005). Dichlorvos acts mainly by irreversibly inhibiting the enzyme acetylcholinesterase (AChE) at cholinergic junctions of the central nervous system (Petroianu et al., 2006) which induce oxidative stress and results to hepatotoxicity in rat (Gupta et al., 2005) Liver plays a crucial role in detoxification of harmful chemical substances, it is the site of biotransformation of many toxic compounds into less harmful products thereby

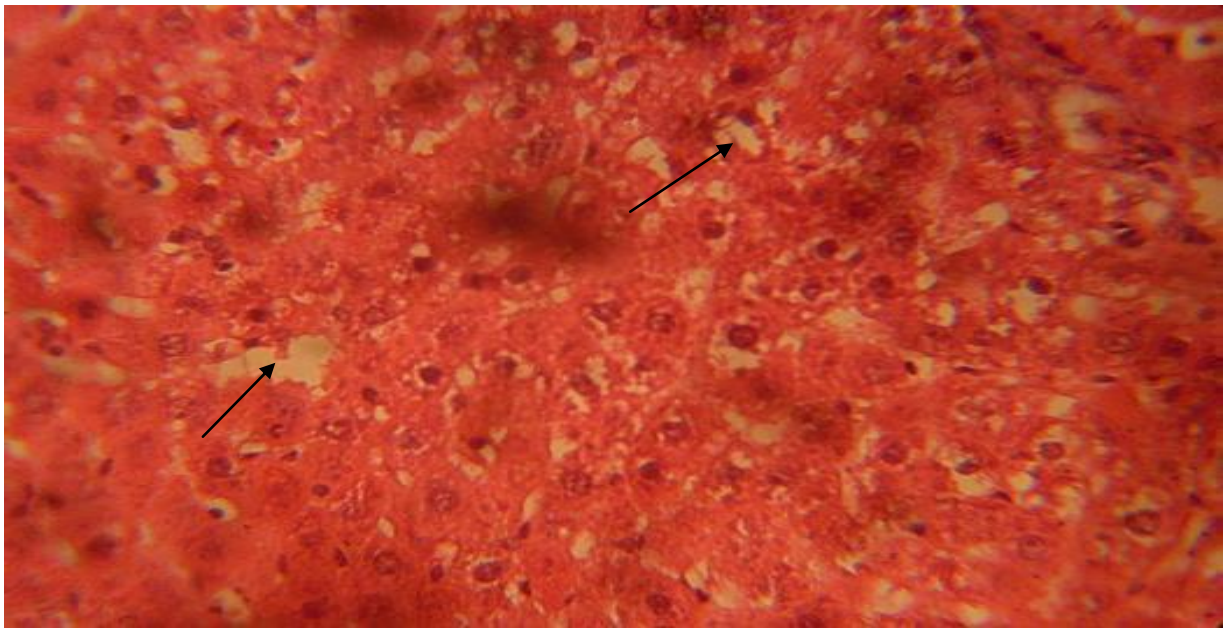

Figure 4. Liver histopathology of Wister albino rat (14.8 mg/kg dichlorvos). ×200 magnification
Observation: Liver tissue showing congestion, fatty changes and steatosis around the central vein.

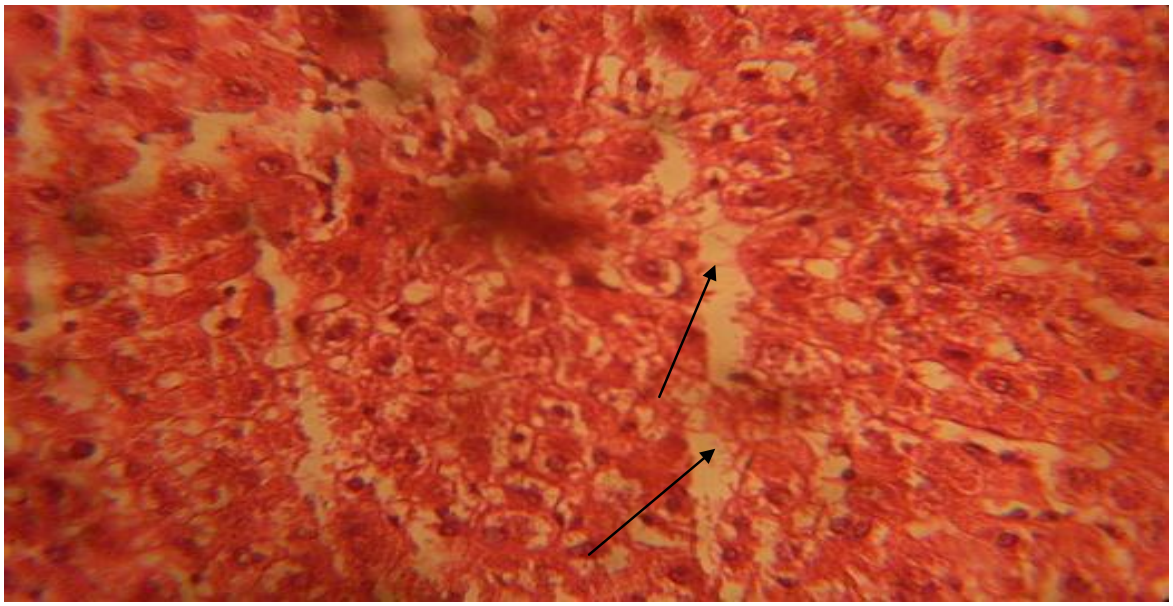

Figure 5. Liver histopathology of Wister albino rat (18.5 mg/kg dichlorvos). ×200 magnification.
Observation: Liver tissue showing centrilobular necrosis and sinusoidal dilation.

reducing their toxicity and ensuring structural stability of the body. Haematological parameters serve as important indices in the monitoring and management of health status. The lifespan of the normal red cell is 120 days, in severe haemolysis the cells survive for only few days, increased bone marrow activity may compensate temporarily for this reduction. However, when the bone marrow fails to increase the production of erythrocytes the offset for the loss anaemia develops (Lewis et al., 2001).

In the present study, acute intraperitoneal administration of varying doses of dichlorvos to the experimental rats caused anaemia as shown by the decreased levels of

haemoglobin, packed cell volume and red blood cells. The observed decreased levels of these parameters were dose dependent, the higher the concentration of dichlorvos the more its impact on the blood parameters. This finding indicates a state ofanaemia which could arise as a result of excessive destruction of erythrocytes by dichlorvos at a rate that exceeds the bone marrows capability to compensate or offset for the blood loss. These findings corroborated with those reported by Mohssen (1997) and Dede and Chike (2000). Erythrocytopenia observed in this study could be attributed to the suppressing effect of dichlorvos on erythropoiesis. The toxicity of dichlorvos on haemopoietic cells in the bone marrow could be due to metabolites of dichlorvos that are produced in relatively high concentration and act in synergistic manner to disrupt the mechanism that regulate blood cell formation. Also obtained was a dose dependent increase in the values of white blood cells and platelets count. The increase in the total WBC could be attributed to the rats' defense mechanism in response to the invading xenobiotic (dichlorvos).

The liver slides in this study as presented in Figure 1 to 5 revealed areas of fatty changes, steatosis, congestion, centrilobular necrosis, sinusoidal dilation and infiltration of the sinuses by few mononuclear cells. Similar observation has also been reported in dichlorvos treated rats in the study by Luty et al. (1998). In the present study, hepatocellular damage was more pronounced at higher doses of dichlorvos (11.1 to 18.5 mg/kg) with no effects observed at lower doses of dichlorvos poisoning and also in the control group. Study also conducted by Binukuma et al. (2010) reported considerable morphological alterations in the structure and function of the liver in male albino rats exposed to chronic dose of dichlorvos. Somia and Madiha (2012) in their study also reported abnormal size and shape of hepatic cells, massive aggregation of inflammatory cells in the portal area and hepatocytomegalocytosis in liver of mice fed for three months on faba beans treated with dichlorvos

Conclusion

This present study demonstrated the acute toxic effects of dichlorvos on haematological parameters and histology of the rats at different dose levels. Acute exposure of the albino rats to dichlorvos poisoning induced anaemia as shown by the decrease levels of HB and PCV was observed in the current study. There was also a significant increase in the total WBC and platelet counts that was dose dependent. Furthermore, dichlorvos promoted necrosis of the liver tissue, thereby altering the normal physiological functions of the liver. From the analysis of the parameters undertaken, it can be inferred that dichlorvos is hepatoxic and also caused anaemia in the exposed rats. Prompt recognition of poisoning cases and aggressive treatment of acute intoxication is essential in order to minimize the morbidity and mortality that could arise from the use of this lethal chemical.

Conflicts of interest

The authors declare that they have no conflicts of interest.

REFERENCES

Alavanja MCR, Hoppin JA, Kamel F (2004) Health Effects of Chronic Pesticide Exposure: Cancer and Neurotoxicity. Ann. Rev. Public Health 25:155-197.

Binukumar BK, Amanjit B, Ramesh K, Aditya S, Kiran DG (2010). Mitochondrial Energy Metabolism Impairment and Liver Dysfunction Following Chronic Exposure to Dichlorvos. Toxicology 270:77-84.

Dede EB, Iyaniwura TT, Salawo OA (1991). Effects of Pre-exposure of Aldrin on Dichlorvos Toxicity in Mice. Niger. J. Neuro-Sci. 1(1):97-106.

Dede EB, Chike CPR (2000). A Study of the Effect of Dichlorvos on the Liver and Small Intestine Using Histopathological and Enzyme Assay Methods. J. Appl. Sci. Environ. Manag. 4(2):33-36.

Gupta SC, Siddique HR, Saxena DK, Kar Chowdhuri D (2005). Hazardous Effects of Organophosphate Compound, Dichlorvos in Transgenic Drosophila Melanogaster (hsp70-lacZ): Induction of hsp70, Antioxidant Enzymes and Inhibition of Acetyl-cholinesterase. Arch. Biochem. 17(25):81-92.

Khan SM, Sobti RC, Kataria L (2005). Pesticide Induced Alteration in Mice Hepato-oxidative Status and Protective Effects of Black Tea Extract. Arch. Clin. Chem. 358:131-138.

Lewis SM, Bain BJ, Bates I (2001). Practical Haematology, Dacie and Lewis, 9th Edition. Churchill Livingston, Edinburgh.

Luty S, Latuszyriska J, Halliop J, Tochman A, Obuchowska D, Przylepa E, Korczak E, Bychawski E (1998). Toxicity of Dermally Absorbed Dichlorvos in Rats. Ann. Agric. Environ. Med. 5:57-64.

Michael E, Nick AB, Peter E, Andrew HD (2008). Management of Acute Organophosphorus Poisoning. Lancet 371(9612):597-607.

Mohssen M (1997). Inhalation Toxicity of Thimet (Phorate) In Male Swiss Albino Mouse, *Mus Musculus*:1. Hepatoxicity. Environ. Pollut. 96(3):383-388.

National Research Council (NRC) (1985). Guide for the Care and Use of Laboratory Animals. A report of the Institute of Laboratory Animal Resources Committee on Care and Use of Laboratory Animals. NIH Pub. No. 85-23. Washington, D.C.: U.S.

Petroianu GA, Hasan MY, Nurulain SM, Shafiullah M, Sheen R, Nagelkerke N (2006). Ranitidine in Acute High-dose Organophosphate Exposure in Rats: Effect of Time-point of Administration and Comparison with Pyridostigmine. Basic Clin. Pharmacol. Toxicol. 99:312-316.

Somia EM, Madiha F (2012) Pathological Effects of Dichlorvos and Fenitrothion in Mice. J. Res. Pract. 208:286-291.

Yurumez Y, Durukan P, Yavuz Y (2007). Acute Organophosphate Poisoning In University Hospital Emergency Room Patients. Int. Med 46(13):965-969.

World Health Organization (2002). The World Report 2002. Reducing Risk, Promoting Healthy Life. World Health Organization, Geneva.


Journal of Toxicology and Environmental Health Sciences
Related Journals Published by Academic Journal
Journal of Clinical Pathology and Forensic Medicine
Journal of Infectious Diseases and Immunity
Journal of Clinical Virology Research
Clinical Reviews and Opinions
Medical Case Studies
Medical Practice and Reviews
academicJournals