ABSTRACT NO.: 1314

CYTOGENOTOXICITY AND OXIDATIVE STRESS IN *CLARIAS GARIEPINUS* (BURCHELL, 1822) EXPOSED TO SIVLER AND COPPER OXIDE NANOPARTICLES, AND THEIR MIXTURE

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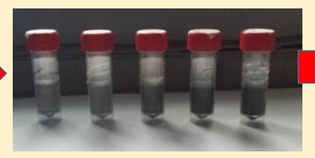
A poster presented at the 56th International Conference of the Society of Toxicology (SOT) held at Baltimore, Maryland ,USA (12 - 16 March, 2017).

INTRODUCTION

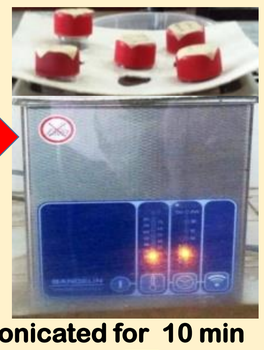
- Silver (Ag) and copper oxide (CuO) nanoparticles (NPs) are metal and metal oxide nanoparticles that are widely used in numerous products particularly because of their antimicrobial and thermo-physical properties respectively.
- Due to their increasing utilisation in various applications, their presence and co-exposure in the aquatic environment may lead to contamination and adverse health effects.
- In this study, we investigated the cytotoxicity and genotoxicity of AgNPs, CuONPs and their mixture using the micronucleus (MN) assay; analysis of haematological parameters in juvenile *Clarias gariepinus.* Also, possible mechanism of damage was assessed using hepatic oxidative stress biomarkers.

METHODOLOGY

AgNPs (<100 nm), CuONPs (< 50 nm) and 1:1 mixture



Weighed & dispersed in distilled water



Sonicated for 10 min

Vortexed for 5 min

C. gariepinus (25.56 ± 4.79g and13.85±1.06 cm) were exposed to 5 Sub – lethal concentrations (6.25 - 100 mg/L) for 28 days

Juveniles of *C. gariepinus*

Piscine Micronucleus test (Bakare *et al.*, 2013)

- Micronucleus (MN) in peripheral blood erythrocytes
- Other nuclear abnormalities

Haematological analysis

- Packed cell volume (PCV)
- Haemoglobin concentration (Hb)
- Red blood cell count (RBC)
- White blood cell count (WBC)

Oxidative stress analysis

- Reduced glutathione (GSH),
- Superoxide dismutase (SOD),
- Catalase (CAT) and
- Malondialdehyde (MDA)

Schematic representation of the methodology

- Interaction between mixture of AgNPs and CuONPs were calculated according to Katsifis (1996)
- Data were analysed using one way ANOVA (Graphpad prism 5.0)

RESULTS

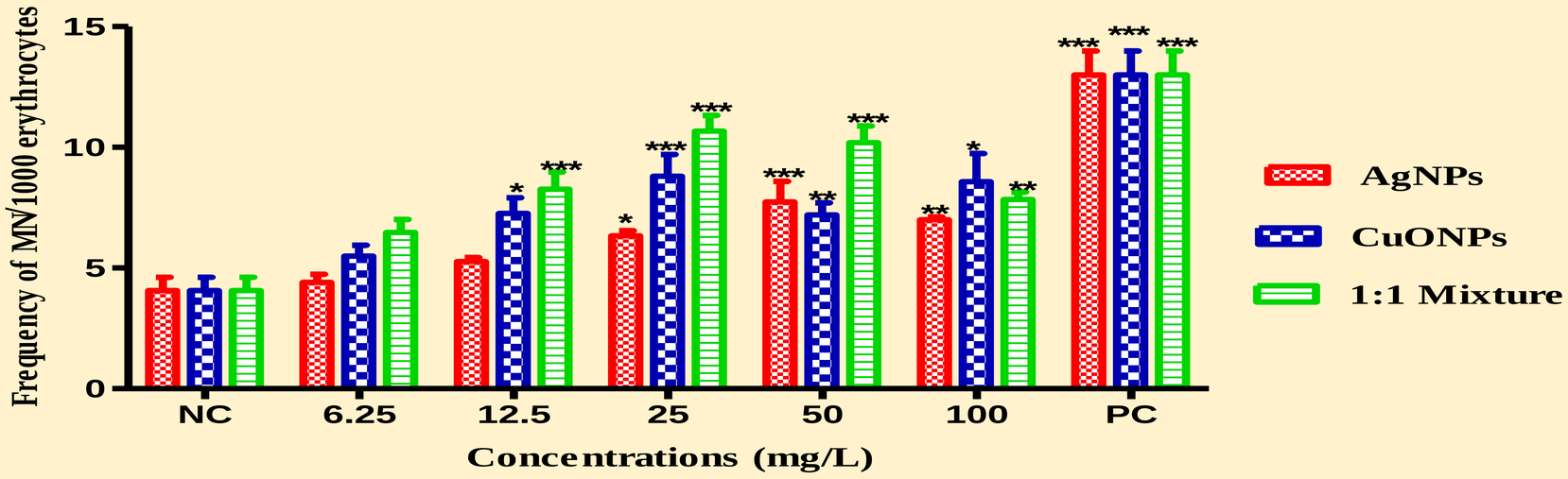


FIGURE 1: Effects of AgNPs, CuONPs and the interaction of their mixture on MN frequencies in peripheral erythrocytes of juvenile *Clarias gariepinus* following 28 days exposure period ; NC: Negative control (dechlorinated tap water), PC: Positive control 0.05mL/L Benzene *,, *** values significantly different from the control group at p<0.05, 0.01 and 0.001 respectively following one way ANOVA**

TABLE 1: Interaction effect of AgNPs and CuONPs mixture (1:1) on MN frequencies in peripheral erythrocytes of juvenile *Clarias gariepinus* after 28 days

Concentrations (mg/L)	**Interaction factor (IF± SE_{IF})
NC	-
6.25	-2.54 ± 0.61
12.50	-1.79 ± 1.31
25.00	-7.72 ± 2.09
50.00	-4.13 ± 2.17
100.00	-4.06 ± 1.57

** NB: Interaction factor IF = (Mixture - Control) -[(AgNPs - NC) + (CuoNPs- NC)] = (Mixture - AgNPs - CuONPs + NC)
- VE IF = antagonistic interaction, +VE IF = synergistic interaction

TABLE 2: Effects of AgNPs, CuONPs and mixture on haematological indices and levels of oxidative stress biomarkers in *C. gariepinus* following 28 days exposure.

Concentrations (mg/L)		Mean ± SD							
		PCV (%)	Hb(g/dl)	RBC (x 10^6/µl)	WBC (/µl))	MDA (unit/mg protein)	GSH (unit/mg protein)	SOD(unit/mg protein)	CAT(µmol H_2O_2 consumed/min/mg protein)
Ag NPs	NC	35.80 ± 2.08	12.32 ± 0.57	3.77 ± 0.10	24190 ± 2966	15.91 ± 2.50	774.3 ± 150.4	0.17 ± 0.01	120.1 ± 43.74
	6.25	38.8 0± 3.34	13.06 ± 1.06	3.92 ± 0.12	21440 ± 1479	7.276 ± 2.39	240.7 ±. 26.05*	0.09 ± 0.01	39.67± 10.93*
	12.5	32.60± 4.13	11.02 ± 1.44	3.38 ± 0.27	21670 ± 1337	28.35 ± 17.1	481.2 ± 144.6	0.08 ± 0.03	119.2 ± 4.10
	25	26.00 ± 3.11	8.44 ± 1.02	2.03 ± 0.40*	12530 ± 1267*	18.22 ± 1.97	912 .0± 224.4	0.37± 0.08***	141 ± 29.40
	50	22.6 0± 2.04*	7.02 ± 0.71*	2.15 ± 0.39*	1585 ± 1234*	24.26 ± 2.37	1127.0 ±166.7	0.30 ± 0.04**	80.28 ± 0.60
	100	29.8 0± 2.03	9.72 ± 0.68	3.20 ± 0.17	13840 ± 1156*	15.65 ± 1.79	778.3 ± 141.4	0.15 ± 0.03	87.06 ±18.09
CuO NPs	NC	35.80± 2.08	12.32± 0.57	3.77± 0.10	24190±2966	15.91 ± 2.50	774.3 ± 150.4	0.17 ± 0.01	120.1 ± 43.74
	6.25	27.20± 1.88	8.88± 0.62*	2.40± 0.47	15910±2044	20.12 ± 2.7	1257.0 ± 136.3**	0.35 ± 0.03***	41.11 ± 4.84**
	12.5	24.40± 0.68*	8.34± 0.25*	2.19± 0.28*	16800±2310	20.42 ± 3.18	1143 ± 130.4*	0.19 ± 0.04	12.06 ± 2.21***
	25	27.60± 2.46	9.32± 0.83	3.04± 0.37	17710±933	37.86 ± 2.5***	845.6 ± 174.8	0.18 ± 0.02	17.25 ± 0.00***
	50	21.20± 0.97*	7.12± 0.27*	1.36± 0.04*	19430±3346	16.23 ±2.2	840 .0± 224.3	0.27 ± 0.02**	3.908 ± 2.22***
	100	28.20± 3.51	9.44± 1.13	2.78± 0.52	17910±1162	29.06 ± 1.25***	1244 .0± 63.1**	0.25 ±0.05*	3.137 ± 2.56***
Mixture	NC	35.8± 2.08	12.32± 0.57	3.77± 0.10	24190± 2966	15.91 ± 2.50	774.3 ± 150.4	0.17 ± 0.01	120.1 ± 43.74
	6.25	23.80± 1.85*	8.08± 0.69*	2.24± 0.33*	16430± 2015*	13.39 ± 2.9	629.5 ± 77.83	0.11 ± 0.07	88 ± 8.93
	12.5	20.80± 1.16*	6.80± 0.29*	1.63± 0.05*	18900± 1778	13.83 ± 3.2	784.3 ± 178.3	0.08 ± 0.02*	80.42 ± 11.39
	25	26.80± 1.07*	8.84± 0.25*	3.34± 0.25	16540± 2482	5.682 ± 0.18*	285.6 ± 79.84*	0.07± 0.03**	53.13 ± 0.00
	50	24.00± 1.05*	7.98± 0.44*	2.25± 0.43*	17160± 1204	14.85 ± 3.97	622.6 ± 162.4	0.13 ± 0.04	50.77 ± 6.42*
	100	21.50± 1.44*	7.25± 0.43*	1.94± 0.27*	14963± 1740*	7.326 ± 1.65*	696.9 ± 240.7	0.08± 0.02*	85.01 ± 20.95

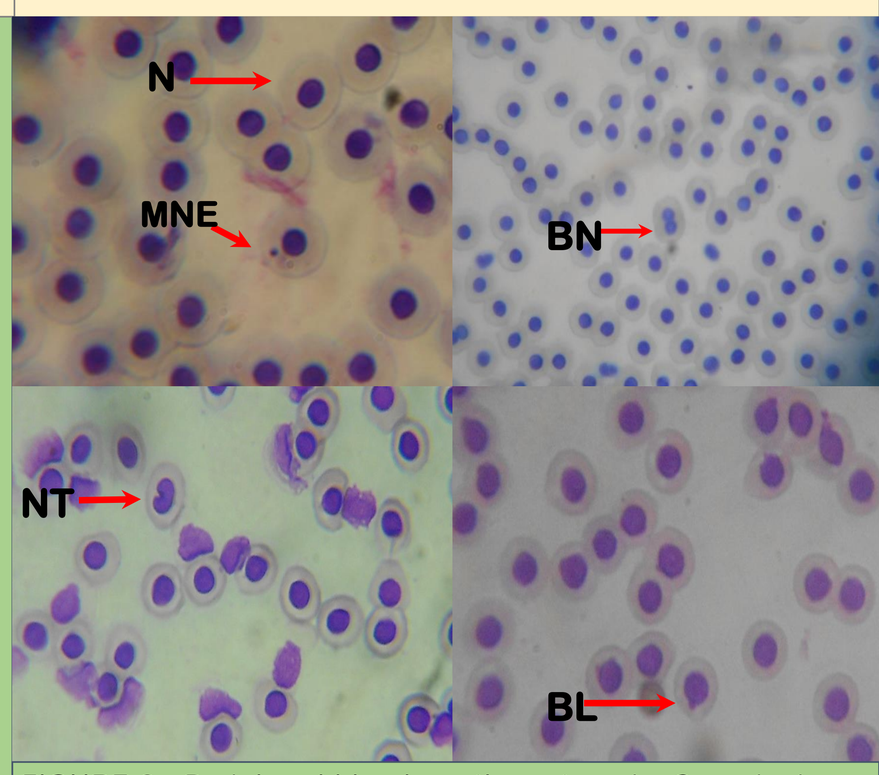


FIGURE 2 : Peripheral blood erythrocytes in *C. gariepinus:* Normal Erythrocyte (N), Micronucleated erythrocytes (MNE), binucleated cells (BN), notched (NT) and blebbed nuclei (BL) in peripheral blood of *C. gariepinus* exposed to AgNPs, CuONPs and their mixtures.

IMPLICATIONS

- The tested NPs and their mixture induced clastogenic and aneugenic chromosome damage which may lead to reduced fitness and embryonic viability, genetic disorders, and loss of aquatic biodiversity.
- The significant decrease in haematological indices indicates hematopoietic suppression of the NPs and their mixture which may affect total physiological processes of the fish.
- Alterations in oxidative stress biomarkers suggest that possible mechanism of DNA damage is via induction of reactive oxygen species (ROS).
- In summary, these results indicate potential human and environmental health risk of the tested NPS.

ACKNOWLEDGMENT: University of Ibadan Postgraduate School for teaching and research assistantship awarded to Ogunsuyi, I. O.

REFERENCES

- Bakare, A. A., Alabi, O. A., Gbadebo, A. M., Ogunsuyi, O.I. and Alimba, C.G. 2013. *In vivo* cytogenotoxicity and Oxidative stress induced by electronic waste leachate and contaminated ground water. *Challenges* 4: 69 – 187
- Katsifis, S. P., Kinney, P. L., Hosselet, S., Burns, F. J. and Christie 1996. Interaction of nickel with mutagens in the induction of sister chromatid exchanges in human lymphocytes. *Mutation Research* 359: 7- 15.