DNA damage and systemic toxicity induced by silver and copper oxide nanoparticles, and their mixture in *Clarias gariepinus* (Burchell, 1822)

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Introduction

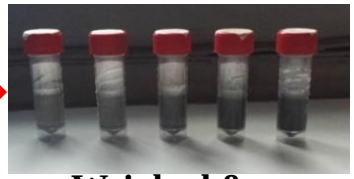
- Nanotechnology industry is a rapidly growing industry, with numerous benefits (Nelson *et al.*, 2016)
- Silver (Ag) and Copper oxide (CuO) nanoparticles (NPs) have unique antimicrobial properties (Makwana *et al.*, 2015).
- AgNPs constitute 24% of NPs containing products (Vance *et al.*, 2015)
- 63 – 91% of ~ 200 metric tons of Cu or CuONPs in 2010 was estimated to have end up in landfill (Keller *et al.*, 2014)

Justification and Hypothesis

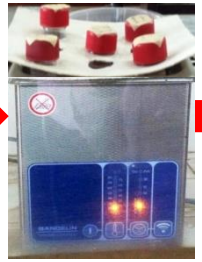
- Rapid increase in manufacture and use of NPs but insufficient studies on potential adverse health effect (Koederith *et al.*, 2014).
- Existing genotoxicity data are largely on the *in vitro* system with limited *in vivo* studies (Pattan and Kaul, 2012).
- Non - existence of information on the *in vivo* impacts of the co - exposure of the selected NPs on aquatic biota
- As part of our ongoing studies on genotoxicity of metal and metal oxide nanoparticle, **We hypothesize that AgNPs and CuONPs and their 1:1 mixture might have DNA damaging and systemic toxicity effect in aquatic model *C. gariepinus*.**

Study Design

AgNPs (<100 nm)
CuONPs (< 50 nm)
1:1 AgNPs + CuONPs



Weighed & dispersed in distilled water



Sonicated for 10 min

Vortexed for 5 min

C. gariepinus were exposed to 5 Sub - lethal concentrations (6.25 - 100 mg/L) for 28 days

Juveniles of *C. gariepinus* (25.56 ± 4.79g and length 13.85±1.06 cm ; n = 270)

Piscine MN assay
(MN and other nuclear abnormalities)

Haematological Analysis
Packed cell Volume (PCV); Haemoglobin concentration (Hb); Red blood cell (RBC); White blood cell (WBC)

Hepatic Oxidative stress analysis
Malondialdehyde (MDA); Reduced Glutathione (GSH); Superoxide dismutase (SOD); Catalase (CAT)

Liver Histopathological Analysis

One way analysis of variance and Interaction factor analysis

Results

- Significant induction of MN which indicates DNA damage
- PCV, Hb and RBC ↓ while WBC ↑
- Presence of histopathological lesions such as diffuse vacuolation of hepatocytes, vacuolar degeneration,atrophy and necrosis livers of exposed fish
- Levels of MDA, GSH and SOD increased while CAT decreased in in the liver
- Interaction analysis of data indicates:
  - antagonistic DNA damage, oxidative damage (MDA), GSH and SOD activities in mixture group while
  - CAT activity was synergistic

RESULTS

Table 1: Effects of AgNPs, CuONPs and the interaction of their mixture on MN frequencies and total nuclear abnormalities (TNA) in peripheral erythrocytes of juvenile *Clarias gariepinus* following 28 days exposure period

	MN Frequency per 1000 erythrocyte (Mean ± SE)			Total Nuclear Abnormalities (TNA) abnormalities per 1000 erythrocytes (Mean ± SE)		
Concentrations (mg/L)	AgNPs	CuONPs	Mixture (Ag + CuO NPs)	AgNPs	CuONPs	Mixture (Ag + CuO NPs)
NC	4.07± 0.55	4.07± 0.55	4.07± 0.55	0.00± 0.00	0.00 ± 0.00	0.00 ± 0.00
6.25 mg/L	4.40± 0.34	5.50± 0.44	6.47± 0.54	0.53± 0.53	0.07 ± 0.00	3.47 ± 1.42
12.5 mg/L	5.27± 0.16	7.27± 0.64*	8.27± 0.71***	1.67 ± 1.29	2.20 ± 0.90	4.20 ± 0.71
25 mg/L	6.33± 0.21*	8.80± 0.90***	10.67± 0.65***	0.00 ± 0.00	0.13 ± 0.13	0.00 ± 0.00
50 mg/L	7.73± 0.86***	7.20± 0.50*	10.20± 0.68***	0.00 ± 0.00	3.53 ± 0.58	1.13 ± 1.13
100 mg/L	7.00± 0.11**	8.58±1.16**	7.83± 0.32***	0.00 ± 0.00	4.08 ± 2.22	0.00 ± 0.00
PC	13.00 ± 0.99***	13.00 ± 0.99***	13.00 ± 0.99***	6.00 ± 3.38*	6.00 ± 3.38*	6.00 ± 3.38*

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**

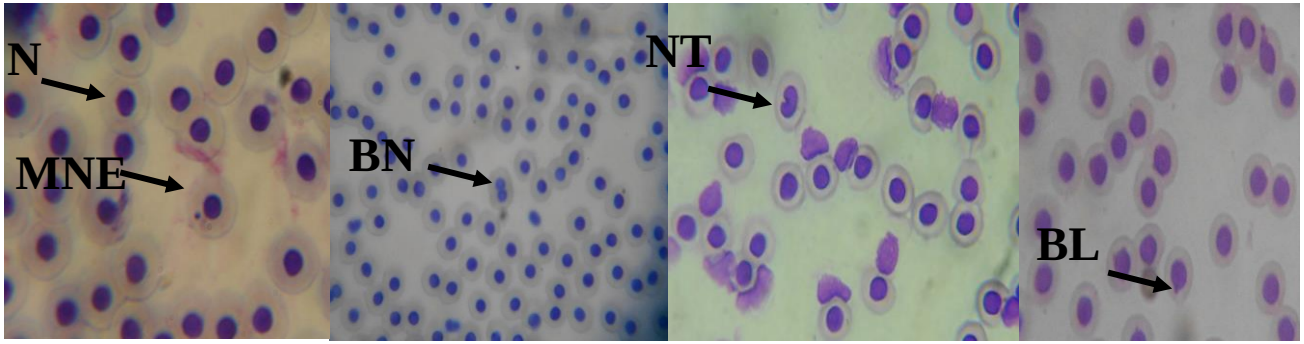


Fig. 2: Normal erythrocyte (N), micronucleated erythrocytes (MNE), binucleated cells (BN), notched (NT) and blebbed nuclei (BL) in peripheral blood of *C. gariepinus* exposed to AgNPs, CuONPs and their mixtures.

RESULTS

TABLE 2: Effect 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					
		RBC (x 10^6/µ/)	WBC (x10^6/µ/))	MDA (unit/mg protein)	GSH (unit/mg protein)	SOD(unit/mg protein)	CAT(µmol H_2O_2 consumed/min/mg protein)
Ag NPs	NC	3.77 ± 0.10	24190 ± 2966	15.91 ± 2.50	774.3 ± 150.4	0.1658 ± 0.01	120.1 ± 43.74
	6.25	3.92 ± 0.12	21440 ± 1479	7.276 ± 2.39	240.7 ±. 26.05*	0.0906 ± 0.01	39.67± 10.93*
	12.5	3.38 ± 0.27*	21670 ± 1337	28.35 ± 17.1	481.2 ± 144.6	0.07609 ± 0.03	119.2 ± 4.10
	25	2.03 ± 0.40*	12530 ± 1267*	18.22 ± 1.97	912 ± 224.4	0.3689 ± 0.08***	141 ± 29.4
	50	2.15 ± 0.39	1585 ± 1234*	24.26 ± 2.37	1127 ±166.7	0.3014 ± 0.04**	80.28 ± 0.60
	100	3.20 ± 0.17	13840 ± 1156*	15.65 ± 1.79	778.3 ± 141.4	0.1526 ± 0.03	87.06 ±18.09
CuO NPs	NC	3.77 ± 0.10	24190 ± 2966	15.91 ± 2.50	774.3 ± 150.4	0.1658 ± 0.01	120.1 ± 43.74
	6.25	2.40± 0.47	15910±2044	20.12 ± 2.7	1257 ± 136.3**	0.3493 ± 0.03***	41.11 ± 4.84
	12.5	2.19± 0.28	16800±2310	20.42 ± 3.18	1143 ± 130.4*	0.1922 ± 0.04	12.06 ± 2.21
	25	2.78± 0.52	17910±1162*	37.86 ± 2.5***	845.6 ± 174.8	0.1789 ± 0.02	17.25 ± 0.00
	50	1.36± 0.04	19430±3346	16.23 ±2.2	840 ± 224.3	0.2747 ± 0.02**	3.908 ± 2.22
	100	2.78± 0.52	17910±1162*	29.06 ± 1.25***	1244 ± 63.1**	0.2523 ±0.05*	3.137 ± 2.56
Mixture	NC	3.77 ± 0.10	24190 ± 2966	15.91 ± 2.50	774.3 ± 150.4	0.1658 ± 0.01	120.1 ± 43.74
	6.25	2.24± 0.33	16430± 2015	13.39 ± 2.9	629.5 ± 77.83	0.1052 ± 0.07	88 ± 8.93
	12.5	1.63± 0.05	18900± 1778	13.83 ± 3.2	784.3 ± 178.3	0.08154 ± 0.02*	80.42 ± 11.39
	25	3.34± 0.25*	16540± 2482*	5.682 ± 0.18*	285.6 ± 79.84*	0.07482 ± 0.03**	53.13 ± 0.00
	50	2.25± 0.43*	17160± 1204*	14.85 ± 3.97	622.6 ± 162.4	0.1349 ± 0.04	50.77 ± 6.42*
	100	1.94± 0.27	14963± 1740*	7.326 ± 1.65*	696.9 ± 240.7	0.08106 ± 0.02*	85.01 ± 20.95

Data are presented as mean ± SD. Values with asterisks (*) indicate significant difference from control at *p < 0.05, ** p< 0.01, ***p< 0.001

RESULTS

TABLE 3: Interaction factor analysis of AgNPs and CuONPs mixture (1:1) on MN frequencies in peripheral erythrocytes and hepatic oxidative stress biomarkers in juvenile *Clarias gariepinus* after 28 days exposure

Conc. (mg/L)	**Interaction factor (IF± SE_{IF})				
	MN	MDA	GSH	SOD	CAT
NC	-	-	-	-	-
6.25	-2.54 ± 0.61	1.904± 2.84	-93.9 ± 88.9	-0.17 ± 0.05	128.22 ± 9.35
12.50	-1.79 ± 1.31	-19.03± 12.37	-65.6 ±152.4	-0.02 ± 0.03	70.16 ± 7.29
25.00	-7.72 ± 2.09	-34.49±1.84	-697.7 ±149.52	-0.31 ±0.05	15.88± 20.79
50.00	-4.13 ± 2.17	-9.73± 2.95	-570.1 ± 186.63	-0.28 ±0.03	87.58 ± 4.73
100.0	-4.06 ± 1.57	-21.47± 1.64	-551.1± 186.99	-0.16 ±0.03	115.81 ±19.62

** NB: Interaction factor IF = (Mixture - Control) -[(AgNPs - NC) + (CuoNPs- NC)] = (Mixture - AgNPs - CuONPs + NC)

- VE IF = antagonistic interaction, +VE IF = synergistic interaction

RESULTS

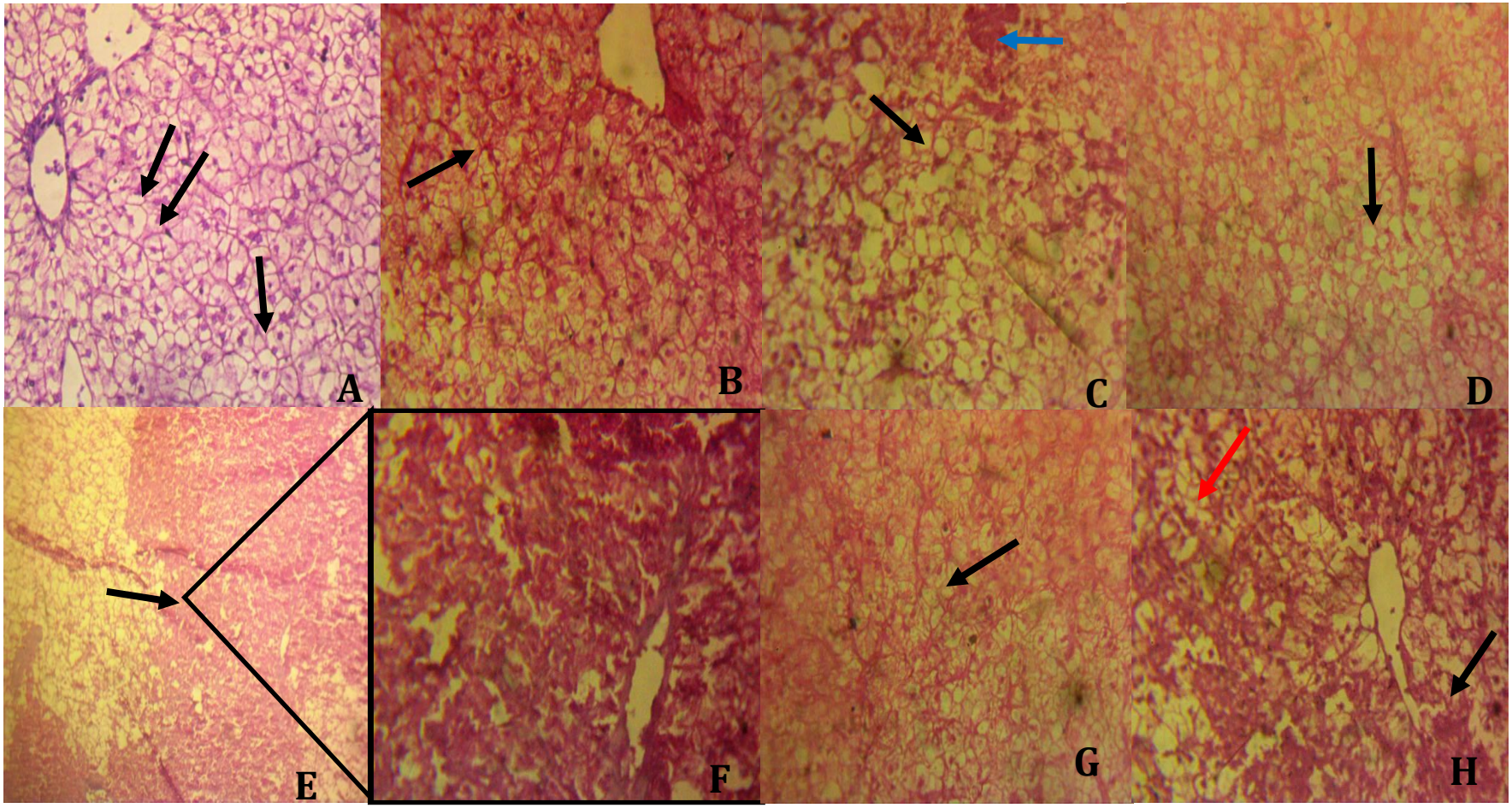


Figure 3: Haematoxylin & Eosin stained sections showing hepatic histological changes in juvenile *C. gariepinus exposed to* AgNPs, CuONPs, 1:1 mixture (B – H) and control

(A) Negative control: the hepatic plates are closely-packed with the hepatocytes (arrows) having large clear cytoplasmic appearance with the nucleus centrally placed; (B) Diffuse vacuolation of hepatocytes (arrows; fat accumulation, normal) (C) Centrilobular vacuolar degeneration (black arrow) of hepatocytes and atrophy of adjacent hepatocytes (blue arrow)(D) Diffuse hepatocellular degeneration and atrophy (arrow) (E-F) Vacuolar degeneration and necrosis of hepatocytes (arrow)(G) Diffuse vacuolar degeneration (red arrow) of hepatocytes and foci of hepatocellular necrosis (black arrow)(H). moderate diffuse swelling of hepatocytes (arrow) Magnification × 400.