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Anti-haemolytic and cytogenotoxic potential of aqueous leaf extract of *Annona muricata* (L.) and its bio-fabricated silver nanoparticles

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Abstract. Nanotechnology is widely gaining worldwide application in biology and medicine because of its proven efficacy. *Annona muricata* contains bioactive phytochemicals with an inherent ability to bio-fabricate metal ions nanoparticles (NPs). *Annona muricata* aqueous leaf extract and its green bio-fabricated silver nanoparticles were evaluated on red blood cells (RBC) for anti-haemolytic activity and cytogenotoxicity on *Allium cepa* cells. The effects of *A. muricata* extract (Am-E) and its biofabricated silver nanoparticles (Am-AgNPs) were observed at 0.7, 7.0 and 70.0 µg/ml on H_2O_2-induced haemolysis in RBC and cyclophosphamide-induced cytogenotoxicity on *A. cepa* cells. Results showed significant and concentration dependent anti-haemolytic activity of Am-E relative to Am-AgNPs. Significant ($P<0.05$) reduction of mitotic index was observed in the groups treated with Am-AgNPs compared with Am-E, which indicates cytotoxic effect of the nanoparticles. The Am-E protected *A. cepa* meristem root cells from cyclophosphamide-induced mitotic repression better than Am-AgNPs. Different degree of chromosomal abnormalities such as chromosome-bridge, sticky chromosome, and c-mitosis were observed in all the treatment groups with chromosome-bridge and sticky chromosome being prominent. This study revealed stronger anti-haemolytic efficacy of Am-E at higher concentrations compared with Am-AgNPs. Chromosomal abnormalities observed in this study suggest greater chromosomal instability as influenced by the nanoparticles compared with the extract on onion cells. The protective effect of the extract against cyclophosphamide-induced chromosomal aberrations may be an indication of its potential as an anti-genotoxic agent.

Keywords: green synthesis, anti-haemolytic, *Annona muricata, Allium cepa,* silver nanoparticles, cytogenotoxicity.

1. INTRODUCTION

Nanotechnology has captured a great scientific interest worldwide due to its wider objectives cum applications in biology and medicine (Shaniba et al. 2017). Its fundamental building block resides in the synthesis of Nanoparticles (NPs) which are products of creation, production, characterization, and manipulation of materials at nano-scale. It enables the amendment of materials at the atomic level with a view to obtain unique properties, which can be annexed for desired applications (Gleiter, 2000).

The distinct optical, electrical, catalytic properties of metal nanoparticles such as Ag, Zn, Pt, Au and Pd, and their roles in biological and pharmaceutical applications are being studied intensively due to their unique amenability (Jacob et al. 2012; Firdhouse and Lalitha, 2015; Shaniba et al. 2017). Silver nanoparticles (AgNPs) have found extensive use in pharmaceutical and cosmetic industries among other metal nanoparticles owing to their broad utility (Sathishkumar et al. 2012; Patil et al. 2017; Annu et al. 2018; Patra et al. 2018). Biological synthesis of AgNPs from natural products viz. bacterial, fungi, yeast and plant extract, and their applications in biology and medicine have tagged them eco-friendly (Lokina et al. 2014; Shaniba et al. 2017; Adebayo et al. 2019a,b).

Annona is a genus of flowering plants of Annonaceae family known for its exotic fruits. Four species of the genus such as *A. muricata*, *A. squamosa*, *A. senegalensis* and *A. cherimola* have been reported to have compelling pharmacological activities (Santos-Sánchez et al. 2018). The pharmacological activities of the genus have been related to considerable quantity of bioactive principles such as phenolic compounds (flavonoids and phenolic acids) (Perrone et al. 2022). *A. muricata* is the one of the most studied species of the genus *Annona* (Santos-Sánchez et al. 2018). *A. muricata* also known as soursoup is a typical tropical evergreen tree with heart shaped edible fruits and it is ubiquitous in most tropical countries (Gavamukulya et al. 2017). Pharmacological and traditional uses of the leaf, bark, root, stem, fruit, and seed extracts include hypoglycemic, anti-cough and analgesic (Hardoko et al. 2015; Coria-Tellez et al. 2018). It has also been found useful as antispasmodic, sedative (Mishra et al. 2013; Moghadamtousi et al. 2015), anti-malarial (Somsak et al. 2016), antioxidant (Balderrama-Carmona et al. 2020), anti-inflammatory (Abdul Wahab et al. 2018) and anticancer (Yang et al. 2015; Najmuddin et al. 2016; Coria-Tellez et al. 2018). It contains phytochemicals such as flavonoids, cardiac glycosides, saponins, alkaloids, tannins, phytosterol, and terpenoids giving it the ability to reduce metal ions (Vijayameena et al. 2013). Previous study from our laboratory have indicated that the physicochemical property of silver nanoparticles synthesized using *A. muricata* aqueous leaf extract is within a normal range (Badmus et al. 2020). The nanoparticles displayed robust biomedical applications such as antidiabetic, antioxidant, antimicrobial and anti-proliferative potential.

Generally, AgNPs have wide applications in household materials, food, pharmaceutical and cosmetic industries. The increase and unregulated disposal of the nanoparticles will elevate environmental availability and bioaccumulation (McGillicuddy et al. 2017). There is a dearth of scientific evaluation of toxicological capability and implication of some identified nanoparticles with strong biomedical presentations. Therefore, this research was designed to study the anti-haemolytic, and cytogenotoxic potential of silver nanoparticles synthesized using an aqueous leaf extract of *A. muricata* on red blood cell and *A. cepa* cell chromosomes respectively.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The leaves of *Annona muricata* were collected from Ologundudu, Ondo State, Nigeria and identified by a taxonomist at the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. A sample of the plant was deposited in Herbarium Unit of the Department with voucher number LHO 250.

2.2 Extract Preparation

Aqueous extraction of *A. muricata* leaf was carried out using the method as earlier reported by Yekeen et al. (2017a) with slight modification. The leaves were pulverized and 6 g of it was soaked in 100 ml distilled water. The soaked sample was heated with continuous stirring for 30 min at 40 °C. The mixture was filtered with Whatman No. 1 filter paper and stored in a refrigerator at 4 °C until use.

2.3 Green Synthesis of Silver Nanoparticles (AgNPs)

A. muricata aqueous extract (1 ml) was added to 40 ml of 1 mM $AgNO_3$ in glass container while 40 ml of Am-E and 1 mM $AgNO_3$ solutions were separately kept in containers as controls. The controls and the reacting mixture of the extract and $AgNO_3$ were placed in sunlight for a complete synthesis of nanoparticles (Yekeen et al. 2017a). A complete change of colour of reacting mixture, an indicator of synthesized nanoparticles was

observed after 30 min.

2.4 Determination of Anti-haemolytic Activity

Anti-haemolytic activities of Am-E and Am-AgNPs were carried out using the method of Joujeh et al. (2017). The blood sample collected from a male Wistar rat through heart puncture was spun at 5000 rpm for 5 min. The plasma was discarded and the precipitate was washed 3 times with phosphate buffer saline (pH 7.4). Five percent of erythrocyte was prepared in phosphate buffer saline. Samples (biosynthesized nanoparticles and the extract) (500 µl) at different concentrations (700, 350 and 175 µg/ml) were added to 1 ml of 5% erythrocyte and incubated for 20 min at room temperature (25 °C). Next, 500 µl of H_2O_2 was added and spun at 5000 rpm for 5 min. The absorbance of free haemoglobin content in the supernatant was read at 540 nm while the percentage inhibitions of H_2O_2-induced haemolysis of the nanoparticles and the extract were calculated using the equation 1.

$$\%\ inhibition\ of\ haemolysis\ = \frac{Abs(Control - Sample)}{Abs(Control)} \times 100 \qquad (1)$$

2.5 Allium cepa *Cytogenotoxicity Assay*

Onion bulbs (240) of approximately the same size were bought at a local market, Owode-Egba, Ogun State, Nigeria. The method earlier reported by Badmus et al. (2013) and Yekeen et al. (2017a, b) was adopted in this study and the experimental set up as described in Table 1. The onions were sundried for two weeks to minimize moisture and aid root growth. The outer scales of the onion bulbs were carefully peeled without affecting the primordial root ring. Fifteen onions were used for each group as indicated in Table 1. The base of each onion bulb was suspended in each container (100 ml beaker) separately containing the control and test solutions at different concentrations. All samples were placed in a dark cupboard at 25 ± 2 °C to reduce the fluctuation of dividing cells. The controls and test solutions were changed at 24 h intervals. Five onions per group were respectively harvested at 48 h and 72 h of growth, and their roots were fixed in ethanol: acetic acid (3:1, v/v) for microscopic evaluation.

Table 1. Experimental Design of Cytogenotoxic Evaluation.

Groups	Treatments
1	Distilled water only (Negative Control)
2	100 µg/ml cyclophosphamide (Positive)
3	0.17 µg/ml $AgNO_3$
4	0.7 µg/ml Am-AgNPs
5	7.0 µg/ml Am-AgNPs
6	70.0 µg/ml Am-AgNPs
7	100 µg/ml cyclophosphamide + 0.7 µg/ml A.m-AgNPs
8	100 µg/ml cyclophosphamide + 7.0 µg/ml A.m-AgNPs
9	100 µg/ml cyclophosphamide + 70.0 µg/ml A.m-AgNPs
10	0.7 µg/ml Am-E
11	7.0 µg/ml Am-E
12	70.0 µg/ml Am-E
13	100 µg/ml cyclophosphamide. + 0.7 µg/ml Am-E
14	100 µg/ml cyclophosphamide. + 7.0 µg/ml Am-E
15	100 µg/ml cyclophosphamide. + 70.0 µg/ml Am-E
16	100 µg/ml of cyclophosphamide. + 1 mM $AgNO_3$

2.5.1 Microscopic Evaluation

The fixed roots were hydrolyzed in 1 N HCl at 65 °C for 3 min. The tip of two roots was squashed on each of the six slides per group and chopped carefully to ease the scoring process. Aceto-orcein was used to stain the prepared slides for 15 min. Five slides were analyzed per group, in which 1000 cells were scored per slide at x1000 magnification for normal and abnormal chromosome behaviour during cell division using various template as earlier reported (Badmus et al. 2013; Yekeen and Adeboye 2013; Yekeen et al. 2017a,b).

2.5.2 Macroscopic Evaluation

After 72 h, the length of the roots of five onions with best growth selected from each of the concentrations was measured with ruler in cm.

2.6 Statistical Analysis

All the data obtained in this study were expressed as mean ± SD. Comparison between treatments was done by analysis of variance (ANOVA) on Statistical Package for Social Sciences (SPSS) 21.0. Software Duncan's multiple range post hoc test was performed to measure variation between the mean with significant difference considered at $p<0.05$.

3. RESULTS

3.1 Biosynthesis of Silver Nanoparticles

The colour change from colourless to brown of the reaction mixture after 30 min exposure to sunlight (UV

Table 2. Anti-haemolytic Activity of Am-AgNP and Am-E.

Concentration (µg/ml)	Am-E (%)	Am-AgNPs (%)
175	65.44 ± 0.6	49.22 ± 0.9
350	61.64 ± 0.6	45.92 ± 0.7
700	49.64 ± 0.8	34.77 ± 1.7

Data were Mean ± SD of triplicate experiments conducted at different time. Am-E (aqueous leaf extract of *A. muricata*); Am-AgNPs (*A. muricata*-fabricated silver nanoparticles).

rays) is an indication of bio-reduction of silver ion to AgNPs

3.2 Anti-haemolytic Activity of Aqueous leaf Extract of A. muricata *and its fabricated silver nanoparticles*

The results in Table 2 show that the bio-fabricated nanoparticle and extract exhibited anti-haemolytic activity in an inverse concentration dependent manner. The activities of the extract and silver nanoparticles were stronger at lower concentration with the extract showing significantly ($P<0.05$) higher activity compared with their biosynthesized silver nanoparticles.

3.3 Effects of Aqueous leaf Extract of A. muricata*, its fabricated silver nanoparticles and cyclophosphamide on root length of* A. cepa

The root lengths assessed after 72 h of exposure revealed that the average root length of the treated groups Am-AgNPs (Groups 4, 5, 6), Cyclo + Am-AgNPs (7, 8 and 9) decreased in a concentration dependent manner and significantly ($P<0.05$) lower than that of the control group. Non-significant ($P>0.05$) increase in the mean root length was observed in the groups treated with 0.7 and 7.0 µg/ml Am-E only (Group 10, 11), while at 70 µg/ml Am-E only (Group 12) significant ($p<0.05$) increase was observed compared to the control group and cyclophosphamide treated group (Group 2) (Table 3). Whereas non-significant ($P>0.05$) difference in average root length was observed in the Cyclo + Am-E (13, 14 and 15) treated groups relative to the Control (Group 1). The mean root length of Am-AgNPs and Cyclo + Am-AgNPs at 0.7 and 7.0 µg/ml decreased significantly ($p<0.05$) relative to the group treated with cyclophosphamide alone. No significant difference was observed between the root lengths of the cyclophosphamide (Group 2) treated group and the control. No growth of roots was observed in the onions treated with $AgNO_3$ alone and Cyclophosphamide + $AgNO_3$.

Table 3. Effects of Aqueous leaf Extract of *A. muricata* and its fabricated silver nanoparticles on *A. cepa* root growth.

Groups	Root length (cm) Mean ±SD
1 (Distilled water only (Negative Control))	1.60±0.80 [a]
2 (100 µg/ml cyclophosphamide (Positive))	1.42±0.50 [a]
3 (0.17 µg/ml $AgNO_3$)	0
4 (0.7 µg/ml Am-AgNPs)	1.57±0.71 [a]
5 (7.0 µg/ml Am-AgNPs)	1.26±0.64 [b]
6 (70.0 µg/ml Am-AgNPs)	0.16±0.07 [b]
7 (100 µg/ml cyclophosphamide + 0.7 µg/ml A.m-AgNPs)	1.58±0.64 [a]
8 (100 µg/ml cyclophosphamide + 7.0 µg/ml A.m-AgNPs)	0.67±0.32 [b]
9 (100 µg/ml cyclophosphamide + 70.0 µg/ml A.m-AgNPs)	0.27±0.13 [b]
10 (0.7 µg/ml Am-E)	1.63±0.71 [a]
11 (7.0 µg/ml Am-E)	1.79±0.98 [a]
12 (70.0 µg/ml Am-E)	2.48±1.14 [b]
13 (100 µg/ml cyclophosphamide. + 0.7 µg/ml Am-E)	1.55±0.68 [a]
14 (100 µg/ml cyclophosphamide. + 7.0 µg/ml Am-E)	1.75±0.74 [a]
15 (100 µg/ml cyclophosphamide. + 70.0 µg/ml Am-E)	1.61±0.73 [a]
16 (100 µg/ml of cyclophosphamide. + 0.71 µg/ml $AgNO_3$)	0

Data are presented as Mean ± SD of triplicate experiment. Mean ±SD with different superscript are significantly different at $P<0.05$. $AgNO_3$: Silver Nitrate, A.m-E: *Annona muricata* Extract, Am-AgNPs: *Annona muricata*- Silver Nanoparticles, Positive control: Cyclophosphamide, Negative control: Distilled water, SD: Standard Deviation.

3.4 Cytogenotoxic effects of Aqueous leaf Extract of A. muricata*, its fabricated silver nanoparticles and Cyclophosphamide on* A. cepa *cells*

The cytogenotoxic effects of Am-AgNP and *A. muricata* extract on *A. cepa* cells are, respectively revealed in Tables 4 and 5 after 48 and 72 h exposure. At 48 h, a concentration dependent reduction in the total number of dividing cells was observed in each of the treated groups compared with the negative control group. The mitotic index (MI) value of the treatment groups was lower than that of the control group whereas, a complete cell growth arrest was observed in groups treated with $AgNO_3$ solution alone and the highest concentration of Am-AgNPs only and in combination with cyclophosphamide. Furthermore, the lesser MI value was observed for both Am-E and Am-AgNPs singly and when combined with cyclophosphamide compared to group treated with cyclophosphamide only. Mitotic index values lesser than the half of the negative control were recorded

Table 4. Cytogenotoxic Effect of *A. muricata* Extract-Mediated Silver Nanoparticles on *Allium cepa* roots meristerimatic cells at 48 h compared with control (positive and negative)

Conc ($\mu g\ ml^{-1}$)	No of Dividing Cells	Mitotic Index (%)	Mitotic Inhibition (%)	Prophase	Metaphase	Anaphase	Telophase	CM	SB	CB	VC	F	No. of A/D	% Aberrant per cell scored
Control	412	8.24	-	227	106	35	44	-	-	-	-	-	-	-
Cyclo														
100	288	5.76	30.10	136	50	35	36	1	16	14	-	-	0.11	0.62
$AgNO_3$														
0.17	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Am-AgNPs														
0.7	303	6.06	26.46	134	47	37	31	-	38	16	-	-	0.18	1.08
7	285	5.70	30.83	127	39	33	38	-	30	18	-	-	0.17	0.96
70	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cyclo + Am-AgNPs														
100 + 0.7	315	6.30	23.54	125	74	32	47	-	27	10	-	-	0.12	0.74
100 + 7	283	5.66	31.31	147	63	12	16	-	36	9	-	-	0.16	0.90
100 + 70	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Am-E														
0.7	267	5.34	35.19	144	38	33	46	-	1	5	-	-	0.02	0.12
7	249	4.98	39.56	132	46	27	36	-	1	7	-	-	0.03	0.16
70	224	4.48	45.63	105	43	35	37	-	2	2	-	-	0.02	0.08
Cyclo + Am-E														
100 + 0.7	271	5.42	34.22	135	56	27	30	-	16	7	-	-	0.08	0.46
100 + 7	246	4.92	40.29	121	57	21	35	1	11	-	-	-	0.05	0.24
100 + 70	199	3.98	51.70	105	36	15	37	-	-	6	-	-	0.03	0.12
Cyclo + $AgNO_3$														
100 + 170	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Cyclo: Cyclophosphamide, Conc: Concentration, CM: C-mitosis, SC: sticky chromosome, CB: chromosome bridge, VC: vagrant chromosome, F: fragmentation, No. of A/D: number of aberration per dividing cell, Positive control: Cyclophosphamide, Negative control: Distilled water.

for both the 70.0 μg/ml Am-E treated group and in combination with cyclophosphamide. Higher mitotic inhibition values were observed in Am-E, cyclophosphamide + Am-E and cyclophosphamide + $AgNO_3$ treated groups relative to the other groups. The mitotic inhibition values were lower in the groups treated with 0.7 μg/ml of AgNPs and AgNPs + cyclophosphamide relative to the group treated with cyclophosphamide only. The highest percent proportion of prophase was observed in the negative control group whereas the least was observed in the group treated with 70.0 μg/ml Am-E alone and when combined with cyclophosphamide. The 7.0 μg/ml Am-E + cyclophosphamide treated group had higher percentage proportion of prophase compared to the negative control. A reduction was observed in the percentage of metaphase in all the treated groups relative to the negative control group. Anaphase stage was higher in the group treated with 0.7 μg/ml AgNPs relative to the other groups and the control. Telophase in the 0.7 μg/ml AgNPs + cyclophosphamide and Am-E only treatment groups was higher than the control and the other groups, whereas it was higher in the control group than the other treated groups.

At 72 h, reduction in the total number of dividing cells of the treatment groups relative to negative control was observed. Cumulative numbers of dividing cells were lower in the 0.7 μg/ml Am-E treated group than half of the positive and negative control. The MI values at 72 h and 48 h were found to be significantly reduced in the treated groups relative to the negative control group. The reduction in mitotic index values was concentration dependent except in the 0.7 μg/ml Am-E and

Table 5. Cytogenotoxic Effect of *A. muricata* Extract-Mediated Silver Nanoparticles on *Allium cepa* root meristerimatic cells at 72 h compared with control (positive and negative)

Concentration (μg ml^{-1})	No of Dividing Cells	Mitotic Index (%)	Mitotic Inhibition (%)	Prophase	Metaphase	Anaphase	Telophase	CM	SB	CB	VC	F	No. of A/D	% Aberrant per cell scored
Control	244	4.88	-	144	46	17	37	-	-	-	-	-	-	-
Cyclo														
100	207	4.14	15.16	112	35	19	32	-	5	4	-	-	0.04	0.18
$AgNO_3$														
0.17	-	-	-	-	-	-	-	-	-	-	-	-	-	
Am-AgNPs														
0.7	216	4.32	11.48	121	43	11	29	-	1	11	-	-	0.06	0.24
7	142	2.84	41.80	75	29	3	31	-	-	4	-	-	0.03	0.08
70	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cyclo + Am-AgNPs														
100 + 0.7	198	3.96	18.85	100	39	9	34	1	4	11	-	-	0.08	0.32
100 + 7	167	3.34	31.56	78	37	9	16	-	19	8	-	-	0.16	0.54
100 + 70	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Am-E														
0.7	83	1.66	65.98	45	22	9	6	-	-	1	-	-	0.01	0.02
7	200	4.00	18.03	104	36	17	38	1	-	4	-	-	0.03	0.10
70	175	3.50	28.28	95	20	9	43	-	2	6	-	-	0.05	0.16
Cyclo + Am-E														
100 + 0.7	213	4.26	12.70	121	29	24	37	-	-	2	-	-	0.01	0.04
100 + 7	229	4.58	6.15	157	18	20	33	-	-	1	-	-	0.00	0.02
100 + 70	-	-	-	-	-	-	-	-	-		-	-	-	-
Cyclo + $AgNO_3$														
100 + 170	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Cyclo: Cyclophosphamide, Conc: Concentration, CM: C-mitosis, SC: sticky chromosome, CB: chromosome bridge, VC: vagrant chromosome, F: fragmentation, No. of A/D: number of aberration per dividing cell, Positive control: Cyclophosphamide, Negative control: Distilled water.

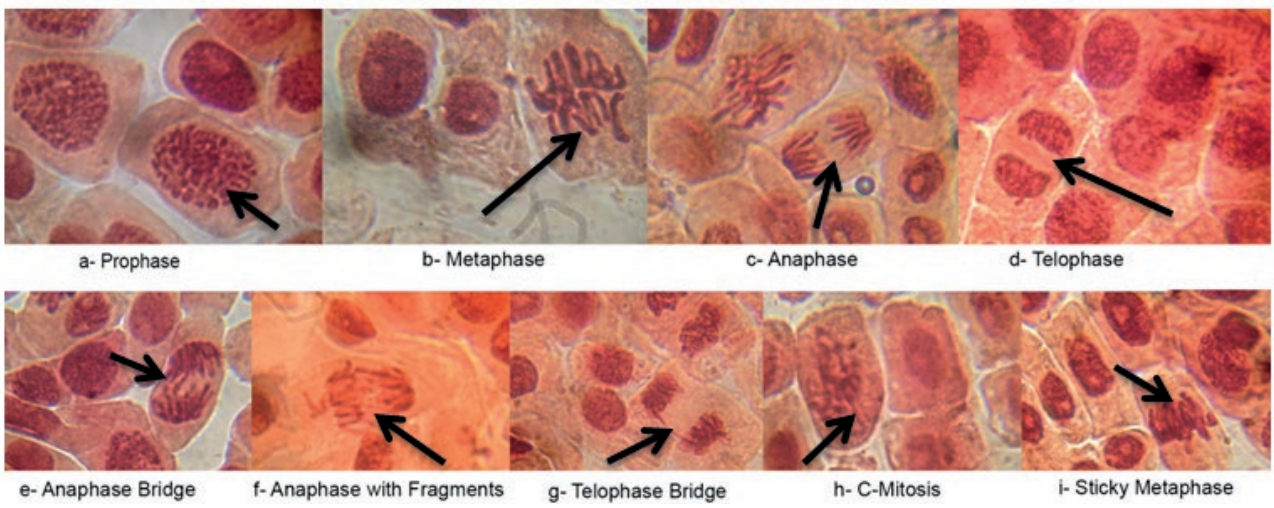

Figure 1. Representative photomicrographs of normal stages of mitotic cell divisions in treated *Allium cepa* root cells and observed chromosomal aberrations.

Am-E+ cyclophospahamide treatment groups where lower values were observed. Complete cell growth arrest was demonstrated in 70.0 µg/ml Am-AgNPs alone, 70.0 µg/ml Am-AgNPs + Cyclo and $AgNO_3$ solution treated groups at 48 h.

Chromosomal aberrations, including chromosome-bridge, sticky chromosome, and c-mitosis were observed in different degrees in all the treated groups with chromosome-bridge and sticky chromosome being prominent in most of the treated groups (Figure 1).

4. DISCUSSION

4.1 Biosynthesis and Characterization of Silver Nanoparticles

A change in colour of silver nitrate solution in the presence of the aqueous leaf extract of *Annona muricata* from colourless to brown that confirms the synthesis of Am-AgNPs nanoparticles has been previously reported (Badmus et al. 2020). Physicochemical characterization results of Am-AgNPs similar to the previous study (Badmus et al. 2020) indicated that the nanoparticles absorbed maximally at 420 nm and FTIR showed that the synthesized silver nanoparticles was possible because of amide and hydroxyl groups of the aqueous leaf extract. Zeta potential of the nanoparticles was -27.2 mV, DLS indicated 86.8 nm size with polydispersity index of 0.329 and XRD/SAED presented crystalline nature of the nanoparticles with face centre cubic (FCC) phase (Santhosh et al. 2015; Gavamukulya et al. 2020; Badmus et al. 2020)

4.2 Anti-haemolytic Effects of Aqueous leaf Extract of A. muricata *and its fabricated silver nanoparticles*

The erythrocyte model for assessing the anti-haemolytic activity of test compounds can reveal the toxicity of an agent and can serve as an indicator of membrane toxicity (Zohra and Fawzia, 2014). Blood cells are easily isolated and the test method using the blood cell can mimic other cell membrane (Farag and Alagawany, 2018). The haemolytic ability of the test compound is proportional to the concentration, chemical constituent and potency of the compound (Zohra and Fawzia, 2014). In this study, the Am-E and Am-AgNPs demonstrated a concentration dependent anti-haemolytic activity. The extract showed a better protection against H_2O_2-induced haemolysis of the red cell membrane compared to Am-AgNPs. This implies that Am-AgNPs is best used at lower concentration because a high concentration as used in this study is toxic to RBC membrane (Raja et al. 2016; Hamouda et al. 2019). Anti-haemolytic activity of plant extract has been shown to be related to the constituent antioxidant agents such as polyphenolic compound (Ramchoun et al. 2015; Karim et. 2020). The bioactive compounds of the plant are responsible for the synthesis of the nanoparticles and also confer the biomedical property such as anti-haemolytic on the synthesized nanoparticles (Kuppusamy et al. 2016; Badmus et al. 2020). The toxic influence of RBC membrane at high concentration by Am-AgNPs could be attributed to the Ag component of the nanoparticles as earlier reported by Choi et al. (2011) and Hamouda et al. (2019) to cause the death of red blood cells, even at low concentration. Clinical outcome of haemolysis can cause anaemia and contribute to blood coagulation abnormalities. However, nanoparticles including sliver have been shown to protect blood against coagulation (Lateef et al. 2018; Elegbede and Lateef, 2019; Azeez et al. 2020). These activities are indicative of biomedical applications of nanoparticles in blood disorder.

4.3 Effects of Aqueous leaf Extract of A. muricata *and its fabricated silver nanoparticles and Cyclophosphamide on root length*

Macroscopic evaluation helps to determine the root sprouting or root growth inhibition effect exerted by the test solution on the onion roots while microscopic evaluation helps to study the harmful qualitative and quantitative effect (cytotoxic effect on onion meristem cells) (Yekeen et al. 2017a). Observations of *A. cepa* root growth inhibition after 48 and 72 h were used as indicator of the cytotoxic nature of Am-AgNPs and Am-E. The extract did not show inhibition of root length, but did protect the roots from cyclophosphamide-induced root length reduction. The protective effect of Am-E on the root growth inhibition imposed by cyclophosphamide and increased mean root length when Am-E was used alone may be credited to the ability of the extract to induce root sprouting (Yekeen et al. 2017a). Contrarily, the Am-AgNPs reduced the root length in a concentration dependent manner attesting to its mitodepressive capability. The reduction of root length was augmented in the presence of both Am-AgNPs and cyclophosphamide at both 48 and 72 h. Root growth inhibition observed in this experiment at both 48 and 72 h exposure to cyclophosphamide together with Am-AgNPs and Am-AgNPs alone is an indication of the genotoxicity nature which can be linked to the presence of heavy metals constituent (Yekeen et al. 2017a). Stunted growth, hardness, and colouration of the roots observed at 72 h

exposure in addition to the aforementioned observations confirm the mitodepressive effect on the onion roots meristem cells.

4.4 Cytogenotoxic Effect of Aqueous leaf Extract of A. muricata *and its fabricated silver nanoparticles and Cyclophosphamide*

The study of chromosome behavior during cell division in order to establish health safety status of a given compound has been the focus of Scientists employing *A. cepa* test. *A. cepa* assay is widely used to study normal and the abnormal chromosome response when the onions base is suspended in a test solution. The assay reveals the effect of a test substance at a minute level of interaction with genetic material, which makes it a robust tool for effective assessment of genotoxic compounds (Bonciu et al. 2018). It is reproducible, sensitive, fast, cheap, and effective in monitoring genetic materials response on exposure to environmental pollution and mutagenic compounds (Badmus et al. 2013; Bhat et al. 2017). Prophase stage of cell division dominated the other cell division stages in all the treated and control groups. An increase in prophase number compared to other stages of cell division has been related to delay in the breaking down of its nuclear membrane (Pankaj et al. 2014). Cell division at the root tip of the onion meristematic region was assessed using mitotic index (Badmus et al. 2013). The reduction in the mitotic index values of the treated groups compared with the negative control revealed the cytotoxicity potential of cyclophosphamide, Am-AgNPs and Am-E at both 48 and 72 h. The reduction of MI by any agent compared with the untreated control is known to relate to cytotoxicity of the tested compound (Asita and Matebest, 2010; Yekeen et al. 2017a). The depression of MI could be linked to the inhibition of DNA synthesis due to the blockage of G_2 phase of the cell cycle, which prevents the cell from entering M-phase during the cell cycle (Badmus et al. 2013; Obute et al. 2016; Yekeen et al. 2017a). Inhibition of mitotic activities is employed for tracing cytotoxic substance (Singh and Roy, 2016). As earlier reported, MI reduction might be as a result of the adverse effects of the extract and Am-AgNPs on the microtubule (Yekeen et al. 2017a). This was corroborated by the total cell arrest obtained when *A. cepa* was treated with $AgNO_3$ and the highest concentration of AgNPs with or without cyclophosphamide. However, the ability of AgNPs to induce cell arrest could be an indication of its capability as an agent of antiproliferation against uncontrolled cell division in cancer cell (Chukwujekwu and Van Staden, 2014). In addition, reduction of mitotic activity in this study could be because of impaired synthesis of nucleoprotein coupled with low level of ATP to power spindle elongation, movement of chromosome and microtubule dynamics (Yekeen et al. 2017a).

The structural changes of chromosome due to an exchange or a break of chromosomal materials are termed chromosome aberration (Preston, 2014). Chromosome aberration (CA) could be as a result of improper or unrepair oxidation of DNA deoxyribose sugar and a nitrogenous base leading to the breaking of the double strand (Badmus et al. 2013). CA observed in cells could be either lethal or viable and can induce somatic or inherited genetic effects (Chang-Hui, 2019). Various chromosomal abnormalities such as chromosome-bridge, sticky chromosome, and c-mitosis were observed in different degrees in all the treatment groups with chromosome-bridge and sticky chromosome being prominent in most of the treatment groups (Figure 1). Kuchy et al. (2016) reported that bridge formation could be linked to chromosome breaks, stickiness, or a reunion of already broken ends of chromosomes. Olorunfemi et al. (2012) reported that sticky chromosome effect is irreversible and ultimately result in cell death. Therefore, total growth inhibition observed with $AgNO_3$ and the highest concentration of Am-AgNPs with or without cyclophosphamide treatment may be due to sticky chromosome formation. The total root growth inhibition as observed in $AgNO_3$ treated group shows that the presence of Ag in Am-AgNPs is responsible for root inhibition and chromosomal aberration observed in Am-AgNPs treated groups.

5. CONCLUSION

The green fabricated NPs using plants have been shown by several studies to have robust biomedical applications. Their actions have been linked to increase surface area due to the reduced size. This study established the anti-haemolytic activity of Am-E and Am-AgNPs. Am-E demonstrated a better anti-haemolytic activity relative to Am-AgNPs at tested concentrations suggesting the toxic potential of biosynthesized AgNPs to RBC at high concentration. The cytotoxicity of Am-AgNPs was revealed through reduction of MI value and increased root growth inhibition of the treatments, suggesting the possibility of employing the biogenic particles as anti-proliferative agent in cancer study. Induction of CA observed at both 48 and 72 h in this study shows the genotoxic potential of both Am-E and Am-AgNPs. While considering the possible influence of Am-AgNPs in disease therapy, its cytogenotoxic potential should

be robustly evaluated before its exposure to human. In addition, there should be restrain in disposing any synthesized nanoparticles into the environment because their toxicity could be far reaching at high concentration.

GEOLOCATION INFORMATION

The research was carried out in Ogbomoso (210214), Oyo State, Nigeria.

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