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Clastogenic and cytotoxic effects of aerial parts' aqueous extract of *Synedrella nodiflora* (L.) Gaertn. on Wistar rat bone marrow cells

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Abstract. *Synedrella nodiflora* is a traditionally used medicinal plant. The aim of the present study was to analyze the clastogenic and cytotoxic effects (CCEs) of aerial parts' aqueous extract of *S. nodiflora* (AAESN) on Wistar rat bone marrow cells (WRBMCs). The CCEs of AAESN were analyzed with light and fluorescence microscopes respectively. The data indicate a dose-dependent (100-500 mg/kg body weight [bw]) increase in the aberrant cell frequencies, chromosomal structural aberrations and a significant ($p<0.001$) increase in apoptosis in the AAESN treated WRBMCs. The chromosomal aberration per 100 cells (apoptosis %) were calculated as 2.01±0.241 (5.02±1.72), 4.76±0.05 (46.73±2.34), 5.37±0.32 (66.92±2.92) and 6.58±0.14 (76.79±0.73) respectively for the AAESN doses of 0, 100, 300 and 500 mg/kg bw. In conclusion, the AAESN may contain phytochemicals with clastogenic and cytotoxic efficacy on WRBMCs, indicating, having the anticancer as well as carcinogenic potentials. Therefore, it demands further an elaborate study to explore the active principle(s) and a proper care should be taken while it is prescribed in traditional medicine.

Keywords. Apoptosis, Clastogenic, Cytotoxic, Synedrella nodiflora, Genotoxic, Anticancer, Secondary metabolites.

1. INTRODUCTION

Cancer is an important worldwide health problem and the antiproliferative pharmacological activities of plant-derived secondary metabolites appear to explain the anticancer effects (Figueroa-Hernandez *et al.* 2005). A variety of bioactive components were isolated from the different medicinal herbs (Cragg *et al.* 1996). Alkaloids, fixed oils and fats, polyphenols, flavonoids, saponins, glycosides, terpenoids *etc*., having medicinal value, were extracted from a wide variety of plant species (Tamilarashi *et al.* 2000). Many plant-based active compounds act as antitumor and apoptotic cell death inducer in tumors (Sato *et al.* 1994). Acetogenins like uvaribonin, 22-epicalmistrin, and chalcone showed significant antiproliferative activity against a panel of cancer cell lines (Pettit *et al.* 2008). Generally, the cell cycle components are the

prime targets of most of the efficient anticancer agents (Li *et al.*, 2002). The discovery of competent anticancer drugs like vincristine and vinblastine were isolated from *Catharanthus roseous;* Paclitaxel (Taxol®) extracted from *Taxus brevifolia* represent trustworthy proof that plants are potential sources of novel anticancer chemotherapeutic drugs (Cragg *et al.*, 1996). The antiproliferative, clastogenic, and cytotoxic pharmacological activities of plant-derived secondary metabolites appear to elucidate the chemo-preventive or anticancer effects. Therefore, searching for the phytochemicals having the clastogenic and cytotoxic effects (CCEs) are of renewed interest in drug discovery for cancer treatment.

The CCEs of a medicine provide a glimpse into its mechanism of action. A clastogen can cause chromosomal structural alterations like chromatid break, deletion, sister chromatid exchanges, sister chromatid union, dicentric chromosome, acentric fragments, micronuclei *etc.* that subsequently may lead to the various cytotoxic effects including cell killing, apoptosis, and necrosis. Moreover, the medicinal plants having these effects, one can also assume their toxicity risk factor for its indiscriminate use in the traditional therapeutic purpose (Thybaud *et al.* 2007). Cancer chemotherapeutic drugs are generally cytogenotoxic, hence subjected to non-target destruction but non-cancerous cells can revive better than the cancerous one (Choudhury *et al.* 2000; Palo *et al.* 2009). Since these clastogenic chemotherapeutic drugs might enhance the chance of secondary cancer development, dose optimization, target specification, and combination chemotherapy with the other antioxidants are highly recommended (Pandit and Choudhury, 2011). Many anti-cancer agents cause DNA damage at a very high level leading to the cell cycle checkpoint activation and programmed cell death (Helleday *et al.* 2008). Administration of the many plant-derived anticancer agents, including paclitaxel, can affect spindle stability leading to abnormal mitosis and chromosomal aberration (Dumontet and Jordan, 2010).

Synedrella nodiflora (L.) Gaertn. (Family: Asteraceae) is an ephemeral flowering weed. It is indigenous to tropical America and also distributed in India, Malaysia, Bangladesh, China, Japan, and other Indopacific countries (Wiart 2006). In India, *S. nodiflora* leaves are traditionally applied for the remedy of rheumatism. In Ghana, oral application of warm aqueous juice of this plant results in the remedy of epilepsy. In Malaysia, it is used externally as a medicine for the treatment of inflammation, headache, and earache. The leaves are also used for the treatment of hiccup, stomachache, and threatened abortion cases (Rathi and Gopalkrishnan 2005; Rahmatullah *et al.* 2010; Bhogaonkar *et al.* 2011). The toxicological (Olukunle and Abatan 2008; Dutta *et al.* 2012), insecticidal (Rathi and Gopalkrishnan, 2005), larvicidal (Ghayal *et al.* 2010), antibacterial, antioxidant (Wijaya *et al.* 2011), antidiarrhoeal, hypoglycaemic (Zahan *et al.*, 2012), anti-inflammatory properties (Haque *et al.* 2012) of this plant have been reported. Our previous study revealed the antiproliferative activity of the aerial parts' aqueous extract of *S. nodiflora* (AAESN) on root apical meristem cells and Wistar rat bone marrow cells (WRBMCs) as well as the presence of different phytochemicals like alkaloids, flavonoids, terpenoids, tannins, phlobatannins, and saponins in the AAESN (Ray *et al.* 2013b). However, the CCEs of AAESN on the mammalian system has not been well-studied. Thus, the present study is focused on the assessment of the clastogenic and cytotoxic effects of the aerial parts' aqueous extract of *S. nodiflora* on WRBMCs *in vivo* condition. Nabeel *et al.* (2008) described the cytogenetic effect of the aqueous extract of *Arum maculatum* on the Bone Marrow Cells of the Swiss male mice. Some of the parameters recorded by the scientists were also taken into consideration in this study. The novel aspects of this study are that it explored the CCEs of AAESN, a source of future anticancer chemotherapeutic drugs, and raised the question against its indiscriminate use in traditional medicine.

2. MATERIALS AND METHODS

2.1. Chemicals

Colchicine, glacial acetic acid, and methanol were obtained from BDH Chemicals Ltd., UK. EDTA was procured from Gibco, Grand Island, N.Y, USA. Ethidium bromide and acridine orange were purchased from Sigma, St. Louis, M.O., USA and S.D. Fine-Chem. Ltd., Mumbai, India respectively. Other chemicals used in this work were of analytical grade from reputed manufacturers.

2.2. Plant products collection, storage, and extract preparation

Plant aerial parts collection, storage, and extract preparation procedures were described in detail in our earlier report (Ray *et al.* 2013b) and briefly the fresh aerial parts' of *S. nodiflora* were collected from Golapbag campus of The University of Burdwan, taxonomically authenticated by Prof. Ambarish Mukherjee, and the voucher specimen (No.BUGBSC013) is maintained in the Department (Figure 1). The dried and pulverized plant product was boiled in double-distilled water

Fig. 1. Showing the aerial parts (leaf, stem, and flower) of *Synedrella nodiflora* (L.) Gaertn.

(1:10, W/V) in a water bath for 30 min. The extract was allowed to cool to room temperature, filtered by Whatman filter paper No. 1 (Sigma-Aldrich, Inc., St. Louis, MO, USA), and then refrigerated at -20°C for further use. For the measurement of extract value (17.64%w/w) and extract concentration (11.3 mg/mL), 10 mL of extract was kept for evaporation to complete dehydration in a hot air oven at 60 °C.

2.3. Experimental animals

Male Wistar-albino rats (age 4-6 weeks; weight 40–60 g) were purchased from local vendors and maintained in the Departmental animal house (in community cages) at room temperature (25±2°C), controlled illumination (12 h light and 12 h dark cycle), and with standard rat diet and water. The rules of the "Institutional Animal Care and Use Committee" were strictly followed throughout the whole experiment and the required total 24 rats were euthanized with prior approval from the Dissection Monitoring Committee (DMC) of The University of Burdwan (No: R-S/N-1/646, Dated 30-03-2016; Under Ref. No. BU-DMC/2016/01/05(a) Dated 13.07.2016).

2.4. Treatment and clastogenicity analysis

The AAESN (100, 300 and 500 mg/kg body weight [bw]) was injected into the peritoneal cavity of the male Wistar rats (Ray et al. 2013b). Control rat groups were injected an equal volume of double distilled water. At each data point, six rats were used. After 12 h of AAESN injection, colchicine (10 mg/kg bw), a standard metaphase arresting agent, was injected into the peritoneal cavity of the rats irrespective of control and treatment groups for 3 h (Ray et al 2013b). Then the animals were euthanized by cervical dislocation just before the femur bones were dissected out (Ray *et al.* 2013b) and the WRBMCs were fixed in aceto-methanol (1:3) after the required hypotonic (0.56% KCl) treatment. Control group was considered for nullifying the sole toxic effect of colchicine. The detailed procedure of metaphase plate preparation and Giemsa staining procedure were described earlier (Ray et al. 2013b). Briefly, the femur bones were dissected out, the bone marrow cells were collected in 15 mL centrifuge tubes by flushing with pre-warmed (37°C) 2.5 mL of 0.56% aqueous KCl solution with 5 mL hypodermic syringe, the cells were maintained in a hypotonic solution for 30 min at 37°C in a water bath and then the cells were fixed with aceto-methanol (methanol 3 parts and acetic acid 1 part). Metaphase plate preparation was done through flame-drying technique and 2% Giemsa (staining duration: 35 min) was used for staining. The Giemsa stained slides were mounted with a coverslip in synthetic medium and the different types of chromosomal abnormalities like chromatid break, terminal deletion, fragmented chromosome, centric fusion, centromeric association, ring chromosome, chromatid gap, chromosomal association, end to end association *etc.* were scored (Kumpawat *et al.* 2003).

2.5. Fluorescence microscopic cytotoxicity analysis

The acridine orange-ethidium bromide (AO-EB) double staining procedure (Bustillo *et al.* 2009) was used to determine cytotoxic effects of AAESN in terms of early and late phases of apoptosis with the fluorescence microscope by observing changes in nuclear morphology and apoptotic blebbing. It is the established fact that acridine orange can infiltrate in both the live and dead cells while ethidium bromide enters only in dead cells. The AO-EB staining strategy causes color differentiation with blue-filter excitation; the living cells (stained only with acridine orange) give green fluorescence, the early apoptotic cells (permitting limited penetration of ethidium bromide) show green to yellowish nuclei with perinuclear chromatin condensation, the late apoptotic cells show a dark red color with fragmented or condensed chromatin, and the necrotic cells give red color with large nucleus having no condensed chromatin.

Here, the AAESN treatment and WRBMCs collection procedures were same as described for clastogenicity analysis, except, the harvested cells were washed in 1X PBS instead of hypotonic KCl solution. After cen-

trifugation at 1000 rpm for 5 minutes and discarding the supernatant, the precipitates were stained with acridine orange-ethidium bromide mix (conc. 100 µg/mL) in 1:1 ratio for 5 minutes in 2 mL Eppendorf tubes. Then, cells were washed thrice with 1X PBS repeating the centrifugation steps. The cells were resuspended in 100 µl 1X PBS and then 20 µL cell suspensions were taken on grease free slide, the percentages of dead cells (apoptotic vs necrotic) and live cells were scored under Leica fluorescence microscope.

2.5. Scoring and Statistical analysis

All the results were expressed as Mean±SEM. Aberrant cell percentage were analyzed through one way ANOVA (d.f.=11) followed by Tukey-Kramer tests. The differences between the untreated and treated groups for cellular viability and chromosomal abnormalities were analyzed with the 2x2 contingency χ^2-test and were considered statistically significant at $p<0.05$, $p<0.01$ and $p<0.001$.

3. RESULTS

3.1. Clastogenic effects of AAESN

Metaphase chromosomal abnormalities were scored in all the AAESN treated groups of rats and were compared with the untreated controls. The various clastogenic effects were observed in the AAESN treated rats. The number of cell abnormality percentage was calculated as 4.76±0.03, 5.37±0.32, and 6.58±0.14 after AAESN treatment respectively with 100, 300, and 500 mg/kg bw as compared with the control (2.01±0.24 %) (Table 1). Among the different clastogenic effects scored, chromatid break, terminal deletion, centric fusion, centromeric association, ring chromosome, chromosomal association, and an end to end association followed a dose-dependent increase in frequencies. Here, the chromosomal association (0.98±0.01) percentage was found to be the highest frequency of chromosomal abnormality followed by centric fusion (0.89±0.03), fragmented chromosome (0.88±0.01), end to end association (0.86±0.02), centromeric association (0.83±0.01), chromatid break (0.54±0.03), ring chromosome (0.51±0.06), and terminal deletion (0.45±0.02) after the treatment with 500 mg/kg bw of AAESN (Figure 2 and Table S1).

Table 1. Pooled data showing the AAESN induced aberrant cell percentage of WRBMCs.

AAESN treatment Dose (mg/kg bw)	TM	Ab.Cell(%)= (TAC/TMC)x100	
		Range	Mean±SEM (% increase)
00	100	1.72-2.48	$2.01\pm0.24^{\#\$}$
100	96	4.69-4.84	$4.76\pm0.05^{*\#\$}$ (136.8)
300	94	4.81-5.93	$5.37\pm0.32^{*\#\$}$ (167.2)
500	109	6.42-6.86	$6.58\pm0.14^{*\#\sharp}$ (227.4)

*Significant at $p < 0.05$ as compared to the control, # at $p < 0.05$ as compared to the 100 mg/kg AAESN treatment, ♯ at $p < 0.05$ as compared to the 300 mg/kg AAESN treatment, $ at $p < 0.05$ as compared to the 500 mg/kg AAESN treatment by one way ANOVA (d.f.=11) followed by Tukey-Kramer Procedure. bw; body weight, TM; Total no. of metaphases counted for studying chromosomal abnormality, Ab.Cell %; aberrant cell per percentage; TAC; Total number of aberration count, TMC; Total metaphase count.

3.2. Fluorescence microscopic analysis for cytotoxicity

Apoptotic cells were examined under a fluorescence microscope after acridine orange and ethidium bromide (AO-EB) combined staining of WRBMCs. A dose-dependent increase in the apoptotic cells (%) was observed in AAESN treated samples. As compared with the untreated cells, a significantly ($p<0.001$) increased percentages of early and late apoptotic cells, and necrotic cells were observed in AAESN treated WRBMCs. The dose-dependent response was more prevalent in apoptotic cell death than that of necrosis. The maximum percentages (85.40±1.71) of viable cells were counted in the

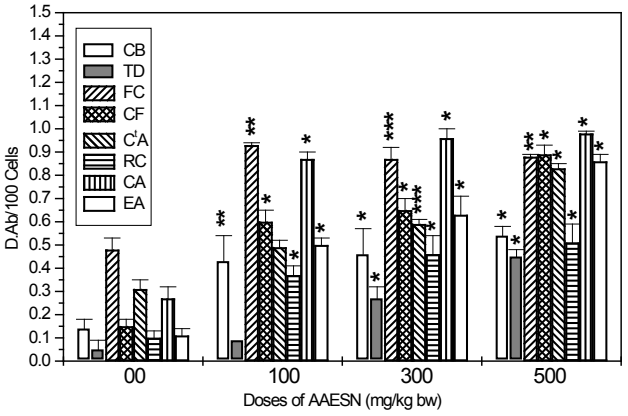

Fig. 2. Influence of AAESN on WRBMCs in terms of different chromosomal abnormalities. *Significant at p<0.05, **at p<0.01 and ***at p<0.001 as compared to the control by 2x2 contingency χ2-test (d.f.=1). The data were represented as Mean±SEM. D.Ab./Cell: Different abnormalities per cell scored; CB: Chromatid break; TD: Terminal deletion; FC: fragmented chromosome; CF: Centric fusion; CtA: Centromeric association; RC: Ring chromosome; CA: Chromosomal association; EA: End to end Association.

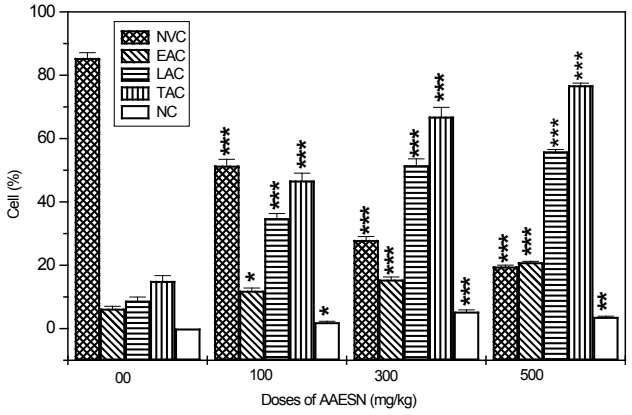

Fig. 3. Shows AAESN induced cytotoxicity observed on WRBMCs under a fluorescent microscope. NVC: normal viable cells; EAC: early apoptotic cells; LAC: late apoptotic cells, TAC: total apoptotic cells. NC: necrotic cells. Experiments were done in three sets and data were represented as Mean±SEM. *Significant at $p<0.05$, **at $p<0.01$ and ***at $p<0.001$ as compared to the control by 2x2 contingency χ^2-test (d.f.=1).

untreated samples and the maximum percentages of early apoptotic (20.92±0.34), late apoptotic (55.87±0.69) and the total apoptotic cell frequency (76.79±0.73) were calculated in AAESN (500 mg/kg bw) treated samples. The maximum percentage (5.34±0.57) of necrotic cells were scored at a dose of 300 mg/kg bw of AAESN for 500 mg/kg of AAESN treatment (Figure 3 and Table S2).

4. DISCUSSION

The leaves of *Synedrella nodiflora* are traditionally used for the remedy of rheumatism, epilepsy, inflammation, headache, stomachache, and earache (Rathi and Gopalkrishnan 2005; Rahmatullah *et al.* 2010; Bhogaonkar *et al.* 2011). In the present study, we have tested *in vivo* CCEs of AAESN on the WRBMCs. The rapidly dividing bone marrow cells are the ideal model to demonstrate antiproliferative activity of herbal extracts (Ray *et al.*, 2013 a, b). The WRBMCs are also considered as a basic model for clastogenicity testing and many authors have used it to study the side-effects of anti-cancer/anti-inflammatory medicines (Pearse *et al.* 2009; Pandit and Choudhury 2011; Haque *et al.* 2012; Ray *et al.* 2013a; Ray *et al.* 2013b; Gomaa 2018). Generally, the bone-marrow mito-depressive drugs show anticancer activity (Prasanthi, 2016). The previous studies indicated the mutagenic activity of pyrethroids on murine bone marrow cells, human peripheral blood lymphocytes, and in aquatic animals (Barrueco *et al.* 1992; Oraby 1997). The general non-steroidal anti-inflammatory drugs (NSAIDs) may also inhibit the proliferation of bone marrow cells without any intervention of hormonal activities in murine models (Chang *et al.*, 2007). The study of the bone marrow suppression is designated as a common toxicity assessment of cytotoxic agents and this toxicity assay had been included in the preclinical study of the four-stage trial system (Heidelberg and Fox, 1990).

Our earlier study indicated a dose-dependent decrease in metaphase frequency (Ray *et al.* 2013b) and in the present study a dose-dependent increase in chromosomal abnormalities (both total and differential counts) and apoptotic cells percentage in WRBMCs. Several anticancer agents put forth their influence through cell cycle events (Salmon *et al.*, 1984). The antiproliferative activities of the several herbal extracts are related to their ability to obstruct DNA synthesis (Akinboro and Bakare 2007; Mercykutty and Stephen 1980). *Toona sinensis* leaf aqueous extract has been reported to have an anti-proliferative influence on human lung cancer cells (Laosinwattana *et al.* 2007, 2009).

In the present study, clastogenicity of AAESN on the WRBMCs revealed that several types of chromosomal abnormalities including chromosomal fragmentation, chromatid break, ring chromosome formation, centromeric association *etc.* were induced by AAESN. Kumpawat *et al.* (2003) showed that raw betel-nut extract introduced clastogenicity on mouse bone marrow cells and human peripheral blood lymphocytes. There are similar kinds of study reports where they explored the genotoxic activity of plant extracts on WRBMCs and human peripheral blood lymphocytes (Pandit and Choudhury 2011; Sakamoto-Hojo *et.al.* 2017).

We previously reported the cytogenotoxic alteration of onion apical meristem cells that were exerted by the aerial parts' aqueous extract of *Ampelocissus latifolia* (Chaudhuri and Ray 2014). Nefic (2008) described the effect of ascorbic acid on human peripheral blood lymphocytes, where vitamin-C at a higher concentration (1,000µg/mL) could induce mitotic arrest and chromosomal abnormalities. The similar kinds of dose-optimization studies were performed using the methanolic extracts of *Artemisia annua* and *Pyracantha coccinea* on *Allium cep*a root apical meristem cells (Karaismailoglu MC 2014, 2017). Pandit and Choudhury (2011) narrated the clastogenic effect of a chemotherapeutic drug on mouse bone marrow cells. Gewirtz (1999) revealed cytogenotoxic effect of anthracyclin antibiotics due to suppression of Topoisomerase-II and thus hindering Topoisomerase-II mediated DNA cleavage and re-ligation. Moreover, it also triggers ROS generation (Dorosho, 1983).

Many anti-cancer agents cause DNA damage at a high level leading to checkpoint activation and programmed cell death (Helleday *et al.* 2008). Administration of anticancer agents like paclitaxel can affect spindle stability leading to abnormal mitosis and chromosomal aberration (Dumontet and Jordan, 2010). One noticeable thing is that colchicine, a mitostatic drug, was also used here to arrest the cells at metaphase but it was equally administered to both control and treatment groups. Here, the treatment group showed a significantly higher level of clastogenic alterations of chromosomes in comparison to the control group in a dose-dependent manner. So, apart from colchicine, there must be extra clastogenic effects of AAESN. We previously reported cell cycle retardation effect as well as the increased prophase-metaphase frequency on *Allium cepa* root apical meristem cells treated by the same extract. Recently, Bonciu *et al.* (2018) used *Allium cepa* root apical meristem cells as a genotoxicity test system. The mito-retarding effect was also noticed in case of WRBMCs in that study in a dose-dependent manner, apart from the influence of colchicine in both control and treatment groups (Ray *et al.*, 2013b). Hence, the strong probability of interaction of the phytochemical(s) present in AAESN with mitotic spindle could not be ignored. However, a detailed mechanism of clastogenic action of AAESN is subjected to further detailed study.

The AO-EB combined staining assay data indicate a dose-dependent increase in the apoptotic cell frequency to a much greater extent than that of necrotic cells. Treatment with AAESN caused the characteristic changes related to apoptotic morphologies in WRBMCs indicating that *S. nodiflora* is an extensive source of natural bioactive substances with apoptotic cell death-inducing activity on WRBMCs. Another important observation was that the necrotic cells' increased frequency did not follow a dose-response relationship and the data, in turn, suggest an apoptotic potential of AAESN. The maximum percentage (5.34±0.57) of necrotic cells were scored at a dose of 300 mg/kg bw of AAESN, indicating, unlike apoptosis, the necrotic cell frequencies did not follow a dose-dependent response pattern.

There are similar reports showing the apoptotic cell death-inducing effects of some of the anticancer agents like isodeoxyelephantopin (Farha *et al.*, 2013) and farnesiferol c (Hasanzadeh *et al.*, 2017). Previously, we also described the cytotoxic effect of aerial parts' aqueous extract of *A. latifolia* on apical meristem cells using AO-EB staining method (Chaudhuri and Ray 2014). Chu *et al.* (2014) showed the antiproliferative and cytotoxic effect of Camptothecin-20(s)-*O*-(2-pyrazolyl-1) acetic ester (CPT6) on breast tumor MCF-7 cells by increased sub G_1 cell population and apoptosis induction among the treatment groups. Farha *et al.* (2013) reported isodeoxyelephantopin (IDOE) mediated apoptosis on nasopharyngeal carcinoma (KB) cells by obtaining more apoptotic morphologies through AO-EB combined staining assay in IDOE treated KB cells. Our results are in agreement with those of Ichikawa who reported the apoptosis-inducing effects of isodeoxyelephantopin in various cells (Ichikawa *et al.*, 2006).

Our previous study revealed the presence of alkaloids, tannins, terpenoids, flavonoids, phlobatannins, and saponins as active ingredients in this extract (Ray *et al.* 2013b). These active ingredients might have interacted with the cell cycle machinery and/or with DNA thus inducing cell cycle delay, clastogenicity, and apoptosis. The AAESN-induced abnormal mitosis in WRBMCs might lead to the mitotic catastrophe through apoptosis and necrosis.

Mitotic catastrophe is an intrinsic mechanism that senses mitotic failure / abnormal mitosis and responds by driving a cell to an irreparable antiproliferative fate of death or senescence (Margaret 2015; Vakifahmetoglu *et al.* 2008). Here, the mitotic catastrophe induced by AAESN was more prone to result in apoptosis rather than necrosis which is more desired outcome in cancer chemotherapy. Our results related to toxicity of this plant are in agreement with the histopathological toxicity and brine shrimp lethality of *S. nodiflora* (Olukunle *et al.* 2008; Dutta *et al.* 2012).

5. CONCLUSION

The phytochemicals present in aerial parts' aqueous extract of *Synedrella nodifolia* could induce cytotoxicity, clastogenicity and mitotic catastrophe in WRBMCs and thus indicate its potential use in cancer chemotherapy in the near future. There is ample scope to explore the active principle(s) of AAESN and the detailed molecular mechanism of the CCEs. Moreover, awareness should be raised among the concerned tribals regarding its probable side-effects due to indiscriminate use and overdose.

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Table S1. Supplementary pooled data of Fig.2 showing the AAESN induced different categories of aberrant cell percentage of WRBMCs.

Doses (mg/kg bw) of AAESN	TM	D.Ab/Cell (%)							
		CB	TD	FC	CF	C^{t}A	RC	CA	EA
00	100	0.14±0.04	0.05±0.04	0.48±0.05	0.15±0.03	0.31±0.04	0.1±0.03	0.27±0.05	0.11±0.03
100	96	0.43±0.11^b	0.09±0.00	0.93±0.01^b	0.60±0.05^c	0.49±0.03	0.37±0.04^c	0.87±0.03^c	0.5±0.03^c
300	94	0.46±0.11^c	0.27±0.05^c	0.87±0.05^a	0.65±0.05^c	0.59±0.02^a	0.46±0.08^c	0.96±0.04^c	0.63±0.08^c
500	109	0.54±0.04^c	0.45±0.03^c	0.88±0.01^b	0.89±0.04^c	0.83±0.02^c	0.51±0.08^c	0.98±0.01^c	0.86±0.03^c

^{a}Significant at p<0.001, ^{b}at p<0.01 and ^{c}at p<0.05 as compared to the control by 2x2 contingency χ2-test (d.f.=1). TM; Total no. of metaphases counted for studying chromosomal abnormality, D.Ab./Cell: Different abnormalities per cell scored; CB: Chromatid break; TD: Terminal deletion; FC: fragmented chromosome; CF: Centric fusion; C^{t}A: Centromeric association; RC: Ring chromosome; CA: Chromosomal association; EA: End to end Association.

Table S2. Supplementary pooled data of Fig. 3 showing AAESN induced apoptosis and necrosis in WRBMCs *in vivo*.

Doses (mg/kg bw) of AAESN	TC	NVC		EAC		LAC		TAC		NC	
		TC	Mean±SEM	TC	Mean±SEM	TC	Mean±SEM	TC	Mean±SEM	TC	Mean±SEM
00	1728	1476	85.40±1.71	102	6.26±0.78	150	8.75±1.23	252	15.02±1.72	0	-
100	2015	1035	51.44±2.00^a	240	11.93±0.89^c	700	34.80±1.54^a	940	46.73±2.34^a	40	2.00±0.33^c
300	1677	468	27.92±1.16^a	258	15.43±0.86^a	861	51.49±2.12^a	1119	66.92±2.92^a	90	5.34±0.57^a
500	2066	404	19.57±0.45^a	432	20.92±0.34^a	1154	55.87±0.69^a	1586	76.79±0.73^a	76	3.68±0.24^b

Conc.: Concentration; TC: total cells; NVC: normal viable cells; EAC: early apoptotic cells; LAC: late apoptotic cells, NC: necrotic cells, TAC: total apoptotic cells ^{a}Significant at *p<0.001* as compared to the control by 2x2 contingency χ^2-test (d.f.=1). Experiments were done in triplicate and data were represented as Mean±SEM.