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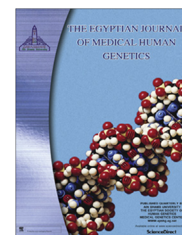

ORIGINAL ARTICLE

# *Terminalia catappa*, an anticlastogenic agent against MMS induced genotoxicity in the human lymphocyte culture and in bone marrow cells of Albino mice

**Mohammad Sultan Ahmad [a,*], Sheeba Ahmad [b], M. Arshad [c], K.B. Rai [a], Mohammad Afzal [d]**

[a] *Department of Zoology, S.N. (PG) College, Azamgarh 276001, U.P., India*
[b] *Department of Zoology, D.S. College, Aligarh, U.P., India*
[c] *Human Molecular Genetics Laboratory, Department of Zoology, Lucknow University, U.P., India*
[d] *Section of Genetics, Department of Zoology, Faculty of Life Science, Aligarh Muslim University, Aligarh, U.P., India*

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**KEYWORDS**

Antigenotoxicity;
*Terminalia catappa*;
Chromosomal aberration;
Sister chromatid exchange;
Replication index

**Abstract** *Background:* *Terminalia catappa* has been used as a folk medicine for treating dermatitis, hepatitis as well as other diseases in India. It possesses anticancer, antioxidant as well as anticlastogenic characteristics.

*Aim:* The aim of the present investigation is to highlight the anticarcinogenic and antimutagenic potential of extracts of *T. catappa*.

*Subjects:* Anticarcinogenic potential of methanolic extract of *T. catappa* has been tested against the carcinogenicity induced by methyl methanesulfonate in the *in vitro* and *in vivo* models.

*Methods:* The parameters for evaluation included chromosomal aberrations (CA), sister chromatid exchanges (SCEs) and replication indices (RI) both in the presence as well as in the absence of exogenous metabolic activation system (*in vitro* study) and total aberrant cells and the frequencies of aberrations were used (for *in vivo* methods).

*Abbreviations:* CA, chromosomal aberrations; SCE, sister chromatid exchanges; RI, replication index; MMS, methyl methanesulfonate; DMSO, dimethyl sulfoxide; ATE, alcoholic *Terminalia catappa* extracts.

* Corresponding author. Tel.: +91 9335753122.
E-mail address: sultansnc@gmail.com (M.S. Ahmad).

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*Results:* Alcoholic extracts of *T. catappa* significantly reduce chromosomal aberration from 34.42%, 70.65% and 82.80% at 24, 48, and 72 h produced by methyl methanesulfonate (MMS) to 22.77%, 49.60% and 42.50% levels. Similarly the number of sister chromatid exchanges was reduced from 6.20 per cell to 3.10 per cell at 48 h of treatment and replication index was enhanced *in vitro* for each concentration and duration of treatment. Further their ameliorating potential was dose and duration dependant. Similarly these extracts significantly reduced the number of aberrant cells or frequency of aberrations per cell *in vivo*.

*Conclusion:* Extracts of *T. catappa* significantly reduced chromosomal aberrations up to 11.65% to 40.30% at different dosages against MMS induced toxicity, similarly sister chromatid exchange was reduced and replication index enhanced *in vitro*. Similarly in the *in vivo* experiments, the effective reduction in clastogeny ranges from 19.70% to 40.90%. Their reducing potential was time and dose dependant.

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## 1. Introduction

*Terminalia catappa* is a native plant of India having smooth gray bark and whorled branches that form its thick canopy. It belongs to the family Combretaceae, and it is widely distributed in tropical and subtropical regions. The leaves of the plant have been used as a folk medicine for treating several diseases in India and in the Philippines. Leaves of *T. catappa* possess anti-cancer, antioxidant as well as anti-clastogenic characteristics and are used in the treatment of different types of cancer, leprosy, eye problems and also for reducing travel nausea, to get rid of intestinal parasites and to stop bleeding during teeth extraction [1,2]. The leaves contain no ascorbic acid and the contents of β-carotene, α-tocopherol and total phenols were found to be 36.7–39.3, 0.94–1.06 and 167–198 mg/g dry weight for the green, yellow fallen and red fallen leaves, respectively [3].

It had been shown in earlier study that the mitomycin C-induced micronuclei in Chinese hamster ovary K1 (CHO-K1) cells were significantly suppressed when the cells were treated simultaneously with the aqueous extract of *T. catappa* leaves [19]. It also reduced 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced production of hydrogen peroxide in human mononuclear leukocytes. These effects were attributed to its ability to inhibit TPA induction of hydrogen peroxide formation and scavenging of free oxygen radicals [4]. Earlier we have shown the antimutagenic and antigenotoxic effects of carotenoids, flavonoids, vitamins and certain plant extracts [5–8]. The aim of present investigation is to highlight the anticarcinogenic and antimutagenic potential of extracts of *T. catappa* in human lymphocytes culture *in vitro* and in bone marrow cells of Albino mice *in vivo*.

## 2. Materials and methods

The whole plants of *T. catappa* were dried under shade at room temperature. The shade dried plants were powdered; around 60 g of coarse powder was defatted with petroleum ether and the contents were extracted exhaustively with 95% methanol at 60 °C. The extract was dried by a vacuum evaporator. Methanolic extract of *T. catappa* was dissolved in dimethyl sulfoxide (DMSO) to prepare different optimum concentrations for studies as shown in Tables A and B.

### 2.1. Table of chemical concentration

(A) Control

| Positive and negative control | Concentrations |
|---|---|
| MMS | 5 μg/ml |
| DMSO | 5 μg/ml |

(B) *In vivo* concentrations of phyto-chemicals

| Phytoproducts | 1st Dose $ATE_1$ | 2nd Dose $ATE_2$ | 3rd Dose $ATE_3$ | 4th Dose $ATE_4$ | 5th Dose $ATE_5$ |
|---|---|---|---|---|---|
| Alcoholic extracts of *Terminalia catappa* (*in vivo* mg/kg.bw) | 200 | 250 | 300 | 350 | 400 |
| Alcoholic extracts of *Terminalia catappa* (*in vitro* μg/ml) | 50 | 100 | 150 | 200 | Nil |

MMS; methyl methanesulfonate, DMSO; dimethyl sulfoxide, $ATE_1$ to $ATE_5$; concentrations of alcoholic extracts of *T. catappa*.

### 2.2. In vivo study

The work is carried out in accordance with the 'The Code of Ethics of The World Medical Association' (Declaration of Helsinki) for Experiments involving humans and animals. Albino mice 8–10 weeks old (25–35 g in weight) were exposed to different test chemicals by appropriate routes (intra peritoneal i.e., I.P. injection) and were sacrificed at sequential intervals of 16, 24, and 32 h of stipulated treatment time. Animals were treated with each test substance as mentioned above. Further processes of slide preparations, cells and chromosomal aberration analyses are adopted from earlier published work [9].

The reduction factors due to test chemical treatments were calculated using the formula published earlier [10].

### 2.3. In vitro lymphocytes culture method

The chromosomal changes (numerical and structural) were utilized for investigation of the genotoxic as well as antigenotoxic

potentiality of test chemicals. The parameters studied included chromosomal aberrations (CA), sister chromatid exchanges (SCEs) and cell growth kinetics (RI) both in the presence and in the absence of exogenous metabolic activation system. The *in vitro* culture methods, preparation of $S_9$ (microsomal fraction), media preparation and analyses of chromosomal aberrations, sister chromatid exchanges, cell cycle kinetics and statistical analysis were followed as per methodology published earlier [9,10].

## 3. Results

### 3.1. In vivo effects

In the present study, the Albino mice were exposed to 16 h of treatments with various doses of alcoholic extracts of *Terminalia* and methyl methanesulfonate simultaneously and it was observed that the percentage of aberrant cells was 10.6%, 9.6%, 8.9%, 8.2% and 7.8%, respectively at five different concentrations of alcoholic extracts of *T. catappa* against the level of 13.2% of aberrant cells induced by methyl methane sulfonate (MMS) alone as positive control. While fragment types of aberrations were most prominent followed by breaks and gaps, exchanges were almost negligible. In terms of percentage reduction in the frequencies of aberrant cells, the observed values are 19.70%, 27.27%, 32.57%, 37.87% and 40.90%, respectively against five different concentrations of alcoholic extracts of *T. catappa*. The maximum effect of *T. catappa* was 41.87% at the 5th concentration of the extract (Table 1 Fig 1).

The effect on the total number of frequencies per thousand cells was 181, 158, 145, 130 and 117 at five consecutive concentrations of alcoholic extracts of *T. catappa* against the count of 237 when treated with MMS alone. The normal values were 25 for distilled water treatment and 28 and 28 for DMSO and alcoholic extracts of *T. catappa* treatment alone (Table 2). When treatment durations were increased to 24 h, the effects of *Terminalia* extracts still followed the same trends of anti-genotoxicity with increased value. These values were 12.0%, 11.2%, 10.1%, 9.4% and 8.3%, respectively for five concentrations of alcoholic extracts of *T. catappa* against 13.5% of MMS treatment only. Normal values were 2.3%, 2.5% and 2.5%, respectively for pure water, DMSO and *Terminalia* extracts alone respectively. The 5th dose of the extract remarkably reduced the percentage of aberrant cells (Fig 1). The total aberrations per thousand cells were 223, 171, 160, 140 and 125 aberrations for extracts of *T. catappa* and MMS, against 286 aberrations due to MMS alone used as positive control. At 32 h of exposure, the percent of aberrant cells observed was 15.0% for MMS alone, and 11.5%, 10.5%, 9.5%, 8.9% and 8.0% for five different concentrations of alcoholic extracts of *T. catappa* plus MMS, whereas the values for normal control was 3.1%. However for DMSO and alcoholic extracts of *T. catappa* alone, the levels were 3.0% and 3.2%, respectively. In terms of the effects on the percent reduction in aberrant cells, the range varied from 11.53% to 38.46%. These values show significant effect of the alcoholic extracts of *T. catappa* on the number and percentage of aberrant cells. It also shows almost dose-dependent relationship. More chromosomal exchange types of aberrations were seen in contrast to the previous two durations of treatment (Fig 1).

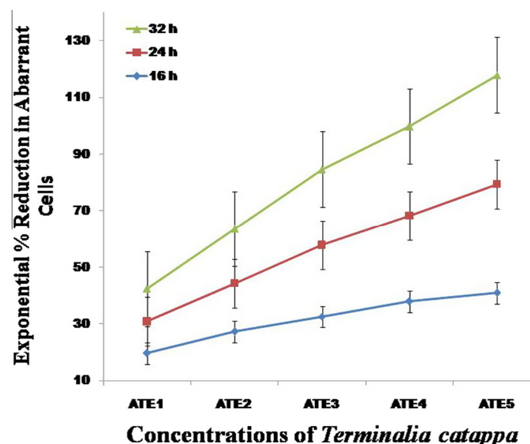


**Figure 1** Showing percentage reduction in aberrant cell due to the effect of alcoholic extract of *Terminalia catappa* at 16, 24 and 32 h of treatment in the bone marrow cells of Albino mice ($p < 0.05$).

**Table 1** Effect of alcoholic extracts of *Terminalia catappa* on the frequency of cells with chromosomal aberrations induced by methyl methanesulfonate (MMS x/kg.bw) at 16 h of treatment.

| Treatment | Termin (Y/Kg.bw) | Cell with pulverized chromosome | Types of chromatic aberrations | | | | Aberrant cell No. (%) | (%) Reduction |
|---|---|---|---|---|---|---|---|---|
| | | | Gaps | Breaks | Fragments | Exchange | | |
| $DH_2O$ | 0 | 00 | 03 | 02 | 21 | 00 | 23 (2.3) | |
| DMSO | 0 | 00 | 01 | 03 | 26 | 00 | 29 (2.9) | |
| MMS | 0 | 15 | 37 | 35 | 79 | 03 | 132 (13.2) | |
| ATE | $ATE_5$ | 00 | 04 | 03 | 23 | 00 | 26 (2.6) | |
| MMS + ATE | $ATE_1$ | 12 | 35 | 36 | 55 | 03 | 106 (10.6) | 19.70 |
| | $ATE_2$ | 10 | 32 | 33 | 51 | 02 | 96 (9.6) | 27.27* |
| | $ATE_3$ | 9 | 31 | 31 | 47 | 02 | 89 (8.9) | 32.57* |
| | $ATE_4$ | 8 | 27 | 30 | 43 | 01 | 82 (8.2) | 37.87* |
| | $ATE_5$ | 6 | 22 | 32 | 40 | 00 | 78 (7.8) | 40.90* |

*Note:* $ATE_1$ to $ATE_5$; concentrations of alcoholic extracts of *Terminalia catappa*, $DH_2O$; double distilled water, DMSO; dimethyl sulfoxide, MMS; methyl methanesulfonate 5 μg/ml/kg body weight) at 16 h of treatment. Calculations were made excluding the gap type of aberration and Y/Kg.bw is the concentration of alcoholic extracts of *Terminalia catappa*.
* Significant at $< 0.05$ probability.

**Table 2** Effect of alcoholic extracts of *Terminalia catappa* on the total number and types of frequency of cells with chromosome aberrations induced by MMS (MMS x/kg.bw).

| Treatment | ATE (Y/Kg.bw) | Cell with aberration | | | | | | | Cell with aberration |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6–9 | |
| $DH_2O$ | 0 | 977 | 21 | 02 | 00 | 00 | 00 | 00 | 25 |
| DMSO | 0 | 971 | 26 | 03 | 00 | 00 | 00 | 00 | 32 |
| MMS | 0 | 868 | 86 | 21 | 09 | 06 | 05 | 05 | 237 |
| ATE | $ATE_5$ | 974 | 24 | 02 | 00 | 00 | 00 | 00 | 28 |
| MMS + ATE | $ATE_1$ | 894 | 69 | 20 | 07 | 04 | 04 | 02 | 181* |
| | $ATE_2$ | 904 | 65 | 18 | 05 | 03 | 03 | 02 | 158* |
| | $ATE_3$ | 911 | 61 | 17 | 04 | 02 | 03 | 02 | 145* |
| | $ATE_4$ | 918 | 60 | 19 | 05 | 03 | 03 | 02 | 130* |
| | $ATE_5$ | 922 | 60 | 19 | 03 | 03 | 02 | 01 | 117* |

*Note:* $ATE_1$ to $ATE_5$; concentrations of alcoholic extracts of *Terminalia catappa*, $DH_2O$; double distilled water, DMSO; dimethyl sulfoxide, MMS; methyl methanesulfonate 5 μg/ml/kg.bw) at 16 h of treatment. Calculations were made excluding the gaps type of aberration and the animals were sacrificed 16 h after MMS treatment 1000 cells from 10 animals were analyzed for each point. Y/Kg.bw is the concentration of alcoholic extracts of *Terminalia catappa*.
* Significant at $<0.05$ probability.

**Table 3** Analysis of chromosomal aberrations after treatment with methyl methanesulfonate along with alcoholic extracts of *Terminalia catappa in vitro* in the presence of $-S_9$ mix.

| Treatments | Durations (h) | Metaphase scored | Percent aberration metaphase | | Aberration/Cell ± SE | | | Aberration/Cell ± SE |
|---|---|---|---|---|---|---|---|---|
| | | | Including gap | Excluding gap | Chromatid | Chromosome | Total | |
| MMS | 24 | 200 | 35.35 | 29.69 | 26.52 | 7.90 | 34.42 | 0.34 ± 0.03 |
| | 48 | 200 | 32.75 | 30.15 | 47.85 | 22.80 | 70.65 | 0.71 ± 0.05 |
| | 72 | 200 | 33.35 | 28.75 | 53.50 | 29.30 | 82.80 | 0.83 ± 0.08 |
| MMS + $ATE_1$ | 24 | 200 | 32.32 | 27.35 | 24.50 | 7.72 | 32.22 | 0.32 ± 0.04 |
| | 48 | 200 | 31.45 | 29.76 | 44.00 | 20.00 | 64.00 | 0.64 ± 0.06 |
| | 72 | 200 | 23.35 | 21.00 | 48.30 | 27.20 | 75.50 | 0.76 ± 0.09 |
| MMS + $ATE_2$ | 24 | 200 | 22.50 | 20.75 | 23.00 | 8.30 | 31.30 | 0.31 ± 0.03 |
| | 48 | 200 | 30.15 | 28.89 | 42.40 | 18.30 | 60.70 | 0.61 ± 0.06 |
| | 72 | 200 | 22.00 | 20.37 | 45.22 | 21.45 | 66.67 | 0.68 ± 0.08 |
| MMS + $ATE_3$ | 24 | 200 | 18.45 | 17.35 | 21.54 | 5.38 | 26.92 | 0.27 ± 0.04 |
| | 48 | 200 | 27.50 | 26.50 | 38.90 | 18.45 | 57.35 | 0.57 ± 0.05 |
| | 72 | 200 | 20.35 | 19.25 | 43.32 | 21.00 | 64.32 | 0.64 ± 0.09 |
| MMS + $ATE_4$ | 24 | 200 | 16.75 | 15.45 | 17.00 | 5.77 | 22.77 | 0.23 ± 0.03 |
| | 48 | 200 | 24.35 | 23.22 | 35.00 | 14.60 | 49.60 | 0.50 ± 0.06 |
| | 72 | 200 | 19.75 | 18.15 | 40.00 | 21.50 | 61.50 | 0.62 ± 0.06 |
| *Control* | | | | | | | | |
| Normal | 72 | 200 | 5.00 | 2.30 | 1.50 | 1.75 | 3.25 | 0.03 ± 0.02 |
| DMSO + $ATE_2$ | 72 | 200 | 4.25 | 3.30 | 2.00 | 1.50 | 3.50 | 0.04 ± 0.02 |

*Note:* SE; standard error, $ATE_1$ to $ATE_4$; concentrations of alcoholic extracts of *Terminalia catappa*, $DH_2O$; double distilled water, DMSO; dimethyl sulfoxide, MMS; methyl methane sulfonate 5 μg/ml culture. Calculations were made excluding the gap type of aberration and significant at $<0.05$ probability level.

The total aberrant chromosomal frequencies per thousand cells recorded were 246 for MMS and about 195, 176, 165, 143 and 130 for alcoholic extracts of *T. catappa* along with MMS alone for five different concentrations of the extracts. These frequencies show the effects of the extracts of *T. catappa* in reducing significantly the total aberrations as well as aberrations per cell.

### 3.2. In vitro effects

The treatment with methyl methane sulfonate (MMS) results in clastogenic abnormalities as observed in percent metaphase aberration, types of aberrations and aberration per 100 cells (34.42%, 70.65% and 82.80%), or being 0.34, 0.71 and 0.83 aberration per cell. For the DMSO plus *T. catappa* (ATE), the values are 0.03, 0.04 per cell at single standard dosage durations. At 24, 48 and 72 h. alcoholic extracts of *T. catappa* bring down these aberrations from 34.42% to 32.22%, 31.30%, 26.92% and 22.77% with four consecutive dosages at 24 h of duration, whereas at 48 h, it brings down the level from 70.65% to 64.00%, 60.70%, 57.35% and 49.60%, done by 1st to 4th concentrations of alcoholic extracts of *T. catappa*. The same trend was noticed, when the treatment duration was increased to 72 h. These values show linear increasing trend

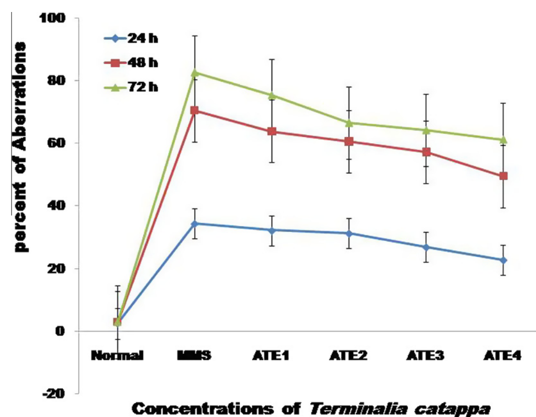


**Figure 2** Expressing the antigenotoxic effect of alcoholic extract of *Terminalia catappa* on chromosomal aberration in the absence of $S_9$ metabolic activation system *in vitro* ($p < 0.05$).

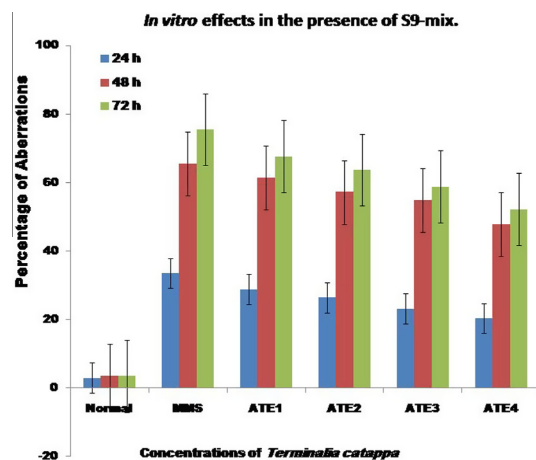


**Figure 3** Expressing the antigenotoxic effect of alcoholic extract of *Terminalia catappa* on chromosomal aberration in the presence of $S_9$ metabolic activation system *in vitro* ($p < 0.05$).

with dosages, but it does not depend on durations. The maximum percentage reductions in the aberrations were produced by highest doses of alcoholic extracts of *T. catappa* (Table 3 Fig 2). When the culture was setup along with metabolic activation system (+$S_9$ mix) the action of MMS showed further increase. The effect of the extracts of *T. catappa* also shows similar trend; they lower the clastogenic activity of MMS. These values show a linear increase with an increase in doses (Fig 3). The highest reduction on clastogeny of cells was noticed at 48 h durations; the other values are also statistically significant.

Sister chromatid exchange counts (Table 4) showed that the reduction is evident both in the absence as well as in the presence of metabolic activation system; there being a lowering trend of the mean range and the total SCEs and SCE per cell respectively from 6.30 to 4.20 and from 6.20 to 3.10. For the analysis of SCE, single treatment duration of 48 h of cultures was used and 50 metaphases were scanned.

The effect of alcoholic extracts of *T. catappa* on replication index (Table 5) shows an elevated level when compared with the MMS treatment i.e. from 1.40 to 1.66 though lower than the normal level of 1.79. The effect, after treatment with metabolic activation system, varied from 1.38 to 1.62, i.e., much effective than without metabolic activation system. Therefore alcoholic extracts of *T. catappa* clearly shows anticlastogenic activities using CA, SCE and RI assays.

## 4. Discussion

Excess generation of ROS can cause oxidative damage to biomolecules resulting in lipid peroxidation, mutagenesis and carcinogenesis [11]. The herbal products are used worldwide in the prevention and treatment of various chronic diseases, and their potential anticancer and antimutagenic effects are under current investigation. *T. catappa* leaf extracts exert a range of biological effects on cells, including antioxidant and hepatoprotective activity on hepatocytes and liver mitochondria, and preventive activity against hepatocyte apoptosis [12]. Studies have also suggested that its protective effects might be related to the scavenging of reactive oxygen species (ROS) [13]. The increased synthesis of very low density lipoprotein-cholesterol observed in fibrosarcoma-bearing control could have led to the increased triglyceride levels in fibrosarcoma-bearing animals. Finally, excessive lipid peroxides formed in fibrosarcoma condition may lead to hyper-lipidemia [14]. Excessive rates of lipid peroxidation may be root of the hyperlipidemia, found in many cancer patients [15]. The treatment of *T. catappa* significantly attenuated the alterations of lipid levels in tissue as well as in serum. The normalization of lipid level in liver and kidney

**Table 4** Analysis of sister chromatid exchange after treatment with methyl methanesulfonate along with alcoholic extracts of *Terminalia catappa in vitro*, in the presence of +$S_9$ mix.

| Treatment | Duration (h) | Metaphase scored | Total | Range | SCE /Cell ± SE |
|---|---|---|---|---|---|
| MMS | 48 | 50 | 310 | 1–11 | 6.20 ± 1.50 |
| MMS + $ATE_1$ | 48 | 50 | 290 | 1–10 | 5.80 ± 1.50 |
| MMS + $ATE_2$ | 48 | 50 | 265 | 1–10 | 5.30 ± 1.50 |
| MMS + $ATE_3$ | 48 | 50 | 185 | 1–9 | 3.70 ± 1.50 |
| MMS + $ATE_4$ | 48 | 50 | 155 | 1–11 | 3.10 ± 1.50 |
| *Control* | | | | | |
| Normal | 48 | 50 | 95 | 0–5 | 1.90 ± 1.00 |
| DMSO | 48 | 50 | 94 | 0–5 | 1.88 ± 1.00 |
| DMSO + $ATE_2$ | 48 | 50 | 97 | 0–5 | 1.94 ± 1.00 |

Note: SE; standard error, $ATE_1$ to $ATE_4$; concentrations of alcoholic extracts of *Terminalia catappa,* $DH_2O$; double distilled water, DMSO; dimethyl sulfoxide, MMS; methyl methane sulfonate 5 μg/ml/ culture. Calculations were significant at <0.05 probability level.

**Table 5** Analysis of cell cycle kinetics after treatment with methyl methanesulfonate along with alcoholic extracts of *Terminalia catappa*, *in vitro*, in the absence of $-S_9$ mix.

| Treatment | Cell scored | (%) Cell in | | | Replication index | 2 × 3 Chi square test |
|---|---|---|---|---|---|---|
| | | $M_1$ | $M_2$ | $M_3$ | | |
| MMS | 200 | 65 | 30 | 05 | 1.40 | |
| MMS + $ATE_1$ | 200 | 57 | 36 | 07 | 1.58 | Significant |
| MMS + $ATE_2$ | 200 | 50 | 40 | 10 | 1.60 | ,,,, |
| MMS + $ATE_3$ | 200 | 49 | 39 | 12 | 1.63 | ,,,, |
| MMS + $ATE_4$ | 200 | 47 | 40 | 13 | 1.66 | ,,,, |
| *Control* | | | | | | |
| Normal | 200 | 37 | 47 | 16 | 1.79 | |
| DMSO | 200 | 38 | 45 | 17 | 1.79 | |
| DMSO + $ATE_2$ | 200 | 40 | 43 | 17 | 1.77 | |

*Note:* 2 × 3 Chi square ($\chi^2$) test, $ATE_1$ to $ATE_4$; concentrations of alcoholic extracts of *Terminalia catappa*, $DH_2O$; double distilled water, DMSO; dimethyl sulfoxide, MMS; methyl methane sulfonate 5 µg/ml/culture. Calculations were significant at $<0.05$ probability level.

tissues and serum upon *T. catappa* treatment may be due to an enhanced lipogenesis or due to decrease in lipolysis, or both. It indicates that alcoholic extract of *T. catappa* exhibited significant reversal of altered lipid levels near to normal values in rats with experimentally-induced, fibrosarcoma [16]. It also showed anti-inflammatory activity in acute and chronic mouse models of 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema [17]. Pretreatment with the *Terminalia* extract also abolished the increase in caspase 3 activity and DNA fragmentation that were observed in the livers of GalN/LPS-treated rats. Free radical formation, specifically hydroxyl and singlet oxygen was seen in GalN/LPS-treated rat liver, these were abolished by pretreatment with the *Terminalia* extract [12].

The ability of the water extract of *T. catappa* to prevent metastasis was investigated under *in vitro* condition, using A549 cell line. The water extract (0–100 µg/mL) did not affect the viability of A549 cells although it was cytotoxic to LLC (Lewis lung carcinoma) cells in a concentration-dependent manner ($IC_{50}$ of 14.5 µg/mL). The invasion and motility of A549 cells was significantly reduced using the aqueous extract (50-100 µg/mL) in concentration-dependent manner. After 24 h, the extract (100 µg/ml) leaves behind only 24.8% and 28.8% of remaining cells, for cell invasion and motility, respectively. Under *in vivo* condition, the water extracts decreased lung metastases of LLC-bearing C57BL/6 mice by 68% compared to controls. After 30 days of treatment with the water extract, there was a 2.6-fold reduction in small solid tumors in tumor-bearing mice as compared to controls. At this time, the tumor weight was reduced by 2.3-fold and there was no apparent signs of toxicity as indicated by body weight monitoring [18]. These results indicate that the aqueous extract of *T. catappa* is a potentially important agent for the prevention of lung cancer metastasis [18]. Similarly, CHO-K1 cells were also protected against bleomycin-induced DNA-strand breaks, measured by the comet assay, when the cells were pretreated with the extract (75 and 100 µg/mL, respectively) for 24 h before exposure to bleomycin (15 mU/ml) for 2 h which is parallel to our finding. These concentrations of the extract were non toxic to CHO-K1 cells as cellular viability was not affected by maximum concentrations of 100 µg/mL of the extract for 24 h. The strong anti-genotoxic effect of the extract was attributed to their ability to ameliorate bleomycin-induced reactive oxygen species formation which was responsible for bleomycin's DNA-damaging effect. The extract suppressed the intracellular formation of superoxides and hydrogen peroxides by bleomycin, probably through direct scavenging of superoxide anions and $H_2O_2$ [19]. Earlier it was noticed that the aqueous extract of *T. catappa* suppressed the growth of H-ras-transformed NIH3T3 cells in a concentration-dependent manner. Cellular growth was completely suppressed by 100 µg/mL of the water extract although in non-transformed NIH3T3 cells, this concentration only produced 30% cell death [20].

The hot water extract of *T. catappa* showed potent short-term chemopreventive action on biomarkers of colon carcinogenesis. Colon cancer was induced in 6 weeks old male F344 rats by weekly subcutaneous (s.c.) injections of azoxymethane (20 mg/kg body weight) for 2 weeks. Aberrant crypt foci are well known as visible preneoplastic lesions that develop in the colonic mucosa of rats treated with azoxymethane, which is a useful biomarker for colon carcinogenesis. *T. catappa* also significantly reduces cell proliferation activity of colonic mucosal epithelium as the proliferating cell nuclear antigen index was lower than that of the control. The protection afforded by *T. catappa* against colon carcinogenesis, therefore, was postulated to be related to its antioxidant activity [21].

## Conflict of interest

We have no conflict of interest to declare.

## Acknowledgements

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