ROLE OF SUSPENDED PARTICULATE MATTER IN ANGIOGENESIS EMPLOYING CROWN GALL TUMOR ASSAY

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INTRODUCTION
Diverse unambiguous carcinogens (classified as Group 1 by IARC) have been the major constituents of the outdoor air pollutants. There exists a wide range of variability in the concentrations of these pollutants, generally, due to their variable sources of generation, geographical location, time of year and weather conditions. The source of pollution can be of natural such windblown dust of particle from different countries, forest fires and volcanic eruptions or manmade such as fumes from vehicles tailpipe exhaust, smoke from burning fossil fuels. In the past decades, the disease burden due to air pollution has been found to be extensively increased affecting masses of people globally.

Introduction of particulate matter (PM) have been the major components of the outdoor air pollutants. There exists a wide range of variability in the concentrations of these pollutants. Generally, due to their variable sources of generation, geographical location, time of year and weather conditions. The source of pollution can be of natural such as windblown dust of particle from different countries, forest fires and volcanic eruptions or manmade such as fumes from vehicles tailpipe exhaust, smoke from burning fossil fuels. In the past decades, the disease burden due to air pollution has been found to be extensively increased affecting masses of people globally.

EXPOSURE TO AMBIENT FINE PARTICLES WAS REPORTED TO CONTRIBUTE 3.2 MILLION PREMATURE DEATHS WORLDWIDE IN 2010 MAINLY FROM CARDIOVASCULAR DISEASE (CVD) AND 2.23 MILLION DEATHS FROM LUNG CANCER. AQUOUS EXTRACTS OF SUSPENDED PARTICULATE MATTER WERE PREPARED USING A MECHANICAL SHAKER FOR 24 HOURS AND THE FRACTURE WAS CENTRIFUGED AND Lyophilized.

RESULTS: MAXIMUM (34.67±1.764) TUMORS WERE INDUCED BY SAMPLES COLLECTED FROM CRYSTAL CHOWK WHEREAS TUMOR INDUCING RATIO (TIR) WAS FOUND TO BE COMPARABLY HIGH FOR TWO SITES VIZ., CRYSTAL CHOWK AND BHANDARI BRIDGE.

CONCLUSION: HIGH TUMOR INDUCING RATIO AT THE ABOVE-MENTIONED SITES WAS CORRELATED TO HIGH TRAFFIC EMISSION FROM AUTOMOBILE EXHAUST. CROWN GALL TUMOR ASSAY HAS BEEN PROVED TO BE RAPID, ECONOMIC AND RELIABLE SCREENING ASSAY FOR ANGIogenesis AGENT.

KEYWORDS: PARTICULATE MATTER, CARCINOGENICITY, TUMOR-INDUCING RATIO

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PM10 was collected on USEPA glass filter paper Type A/C of pore size 1 µm; with a dimension of 20.3 x 25.4 cm² using High Volume Sampler (EnviroTech, APM 415) with constant flow rate (1.2 m³ min⁻¹). The sampling was done over 8 hour period starting at 8.00 AM and ending at 5.00 PM in the evening for three days. 18 samples were collected from six sites. As per CPCB
Guidelines, special attention was given to calibrate the machine, some of the samples were also lost due to electrical failure as well as sampler malfunction during the sampling period.

**Sample extraction and analysis**

The particulate matter holding filter paper were cut into small pieces and put into a flask containing distilled water (DW). It was kept on a mechanical shaker for 24 h and was filtered through filter paper Whatmann No.1. The filtrate was collected and centrifuged at 5000 rpm and the supernatant was collected. The collected supernatant was lyophilized. The lyophilized samples were redissolved in DMSO and were further diluted using distilled water to prepare different concentrations viz., 1, 25, 50, 75, 100, 250, 500 and 1000 (ppm) to analyze effects of aqueous extracts of particulate matter in the initiation of tumors using Crown gall tumour inducing assay.

**Preparation of bacterial culture**

The strain of *A. tumefaciens* with MTCC No. 431 was purchased from IMTECH, Chandigarh. The lyophilized culture (powdered) was provided in the form of the ampoule. The ampoule was broken under the sterilised conditions, and the culture (powder) was poured to 25 ml flask containing 12 ml of nutrient broth. The flask was kept on a shaker for 18 h at a revolution of 180-225 rpm and temperature of 28 °C. The culture was poured to 2 ml vials and was preserved in liquid nitrogen for future use. The culture from the permanent strains was streaked on the nutrient plate and was kept in BOD incubator at 28 °C for 24 h. The culture plates were preserved in a refrigerator at 4 °C for future use. On the day of the experiment, a loopful of culture was taken with the help of inoculation needle and was poured to 25 ml flask containing 12 ml of nutrient broth. The flask was kept on a shaker for 18 h at 180-225 rpm and 28 °C. The solution was used as *A. tumefaciens* culture for further experiment.

**Crown gall tumor inducing assay**

Crown gall tumor inducing assay was performed according to the standard protocol with slight modification [14]. 50 µl of a solution containing TSPM extract and *A. tumefaciens* culture (1: 1) was used for the experiment. Similarly, 50 µl solutions containing 1 ppm lead and *A. tumefaciens* (1: 1) was used as positive control while that of DW/DMSO and *A. tumefaciens* (1: 1) was used as negative control.

**RESULTS AND DISCUSSION**

Tumor assay carried out to evaluate effects of particulate matter extracts revealed a dose-dependent tumor activity in all the studied samples (table 1). The extracts of particulate matter collected from crystal chowk have shown the maximum tumor activity (34.67±1.764) followed by Bhandari bridge (33.00±2.517) in terms of a number of tumors at the highest concentration used (1000 µg/ml). Tumor-inducing ratio (TIR) for these sites was observed to be 3.25 and 3.54, respectively (table 2). The tumor induction was found to be in the order of Crystal chowk>Bhandari bridge>Railway station>Ram bag>Paranji avenue-Garden colony. The tumor-inducing ratio was also found to be dose-dependent manner as depicted in fig. 1. Tumor, not being a phenomenon of normal cells, is an abrupt proliferation of cells which disrupts the neighbouring cells [14]. During this process, excessive proliferation occurs because of continuous supply of nutrients through newly formed channels (blood vessels) and other growth factors resulting in rapid multiplication of tumor cells. However, during crown gall disease of potato, the growth of the tumor is supported via nutrient agar medium resulting in its marked progression. As cancer growth factors are leading challenge for emerging oncologist to combat with this problem, crown gall tumor assay was widely used for assessing anti-angionogenesis activities of several plants/plant products and has exhibited significant results [14-16]. It is well documented that some tumorigenic mechanisms are similar in plants and animals [14, 17].

During the present study, tumor-inducing effects of particulate matter extracts were studied. It was observed that the samples collected from Bhandari bridge and Crystal chowk had shown the significantly high tumor-inducing potential. Both these sites exposed to wide variety of activities including traffic emissions, open barbequres and restaurant chimney exhaust (using hearths) as well as personal experience to exposure of frequent prolonged traffic jams (comprising cars, scooters, diesel driven cars) due to narrow overbridge. Role of DEPs in the progression of angiogenesis was well documented [18]. The present study revealed that the assay used here can be explored in future for rapid screening of mutagens present in the environment due to its easy handling as compared to mammalian assays.

**Table 1: Effects of total suspended particulate matter on induction of tumors following crown gall tumor assay**

| Conc. | No. of tumors (mean±SE) | RS | CC | BB | GC | RA |
|-------|-------------------------|----|----|----|----|----|
| 1     | 1.00±0.50               | 9.33±0.667 | 5.00±0.577 | 1.33±0.333 | 1.00±0.577 |
| 25    | 6.00±1.528             | 10.67±0.333 | 8.00±0.577 | 3.67±0.333 | 1.33±0.333 |
| 50    | 9.33±0.682             | 12.00±0.577 | 12.67±1.202 | 5.00±0.577 | 1.33±0.333 |
| 75    | 11.33±0.553            | 14.67±1.453 | 13.00±1.002 | 3.00±0.577 | 5.00±0.577 |
| 100   | 14.67±1.453            | 15.00±1.732 | 15.33±0.882 | 9.33±0.882 | 4.33±0.882 |
| 250   | 16.33±1.333            | 22.67±0.882 | 24.00±0.577 | 12.33±0.882 | 5.00±0.577 |
| 500   | 20.67±2.186            | 24.67±1.764 | 28.33±1.453 | 13±1.155 | 6.33±0.577 |
| 1000  | 27.33±2.017            | 34.67±2.517 | 32.00±2.517 | 22±1.002 | 7.00±0.577 |
| OC    | 12.67±1.202            | 10.33±0.882 | 9.33±0.882 | 12.67±1.202 | 10.67±0.880 |
| PC    | 24.00±1.732            | 24.67±2.028 | 20.33±1.856 | 21.00±2.082 | 21.67±1.760 |
| F-ratio (9,20) | 36.91*      | 39.25*    | 50.380*    | 44.062*    | 64.702*    |
| HSD   | 6.680                  | 6.610      | 6.510      | 5.254      | 3.880      |

* represents significance level at p≤0.05, RS: Railway station; CC: Crystal Chowk; BB: Bhandari Chowk; RB: Ram Bag; GC: New Garden Colony; RA: Railway avenue; OC: Only culture; PC: Positive control [Pb(C2 H3O2 )2 (1 ppm)]

The tumor induction was found to be in the order of Crystal Chowk > Bhandari bridge > Railway station > Ram Bag > Ranjit Avenue - Garden colony. The tumor-inducing ratio was also found to be dose-dependent manner as depicted in fig. 1. Tumor, not being a phenomenon of normal cells, is an abrupt proliferation of cells which disrupts the neighboring cells [14]. During this process, excessive proliferation occurs because of continuous supply of nutrients through newly formed channels (blood vessels) and other growth factors resulting in rapid multiplication of tumor cells. However, during crown gall disease of potato, the growth of the tumor is supported via nutrient agar medium resulting in its marked progression. As cancer growth factors are leading challenge for emerging oncologist to combat with this problem, crown gall tumor assay was widely used for assessing anti-angionogenesis activities of several plants/plant products and has exhibited significant results [14-16]. It is well documented that some tumorigenic mechanisms are similar in plants and animals [14, 17].

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Table 2: Tumor-inducing ratio (TIR) of different concentrations of total suspended particulate matter following crown gall tumor assay

| Conc. | RS | CC | BB | RB | GC | RA |
|-------|----|----|----|----|----|----|
| 1     | 0.07 | 0.88 | 0.54 | 0.10 | 0.09 | 0.11 |
| 25    | 0.47 | 1.00 | 0.86 | 0.29 | 0.12 | 0.20 |
| 50    | 0.74 | 1.13 | 1.36 | 0.39 | 0.13 | 0.37 |
| 75    | 0.90 | 1.38 | 1.39 | 0.50 | 0.28 | 0.43 |
| 100   | 1.16 | 1.41 | 1.64 | 0.73 | 0.41 | 0.49 |
| 250   | 1.29 | 2.13 | 2.57 | 0.97 | 0.47 | 0.60 |
| 500   | 1.63 | 2.31 | 3.04 | 1.02 | 0.59 | 0.63 |
| 1000  | 2.16 | 3.25 | 3.54 | 1.73 | 0.66 | 0.77 |
| OC    | 1    | 1    | 1   | 1   | 1   | 1   |
| PC    | 1.90 | 2.31 | 2.18 | 1.66 | 2.03 | 1.86 |

RS: Railway station; CC: Crystal Chowk; BB: Bhandari Chowk; RB: Ram Bag; GC: New Garden Colony; RA: Railway Avenue; OC: Only culture; PC: Positive control [Pb(C$_2$H$_3$O$_2$)$_2$ (1 ppm)]

Table 3: Characterization of tumor-inducing ratio (TIR)

| Tumor-inducing ratio (TIR) | Characteristic |
|---------------------------|----------------|
| 0-1                       | Non-toxic      |
| >1-2                      | Moderate toxic |
| >2-3                      | Toxic          |
| >3                        | Highly toxic   |

Fig. 1: Effects of suspended particulate matter on induction of tumors following crown gall tumor assay, RS: Railway station; CC: Crystal Chowk; BB: Bhandari Chowk; RB: Ram Bag; GC: New Garden Colony; RA: Railway Avenue; OC: Only culture; PC: Positive control [Pb(C$_2$H$_3$O$_2$)$_2$ (1 ppm)]; TIR: Tumor-inducing ratio

Potato discs untreated (a) in absence of Agrobacterium tumefaciens and treated with lead (b) and with particulate matter (c) in presence of Agrobacterium tumefaciens
Potato discs treated with lead in the presence of Agrobacterium tumefaciens (d-f)

Fig. 2: Potato discs treated with aqueous extracts of suspended particulate matter in the presence of Agrobacterium tumefaciens (g–l)

CONCLUSION
Our findings indicate the promising role of particulate matter in tumor induction using crown gall tumor assay. This is the first-hand experiment exhibiting the effects of particulate matter in the progression of tumors using a plant bioassay. This assay has been proved to be useful in future studies to further isolate different mutagens/oncogenes/neoplastic molecules present in fractions of urban ambient air so that combating strategies should be formulated before occurring of any ambient episodic mortality.

CONFLICTS OF INTERESTS
Declared none

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