DOI: 10.15415/jptrm.2013.11007

# Cytoprotective Activity of *Adhatoda Vasica Extract* and Vasicine Against Tobacco Smoke Induced Cytotoxicity

**Rachana, Mamta Pant and Sujata Basu**

Department of Biotechnology, Jaypee Institute of Information Technology, Noida, INDIA
E-mail: rachana.dr@gmail.com

**Abstract**

The present study was undertaken to investigate the protection against cytotoxicity due to tobacco smoke by *Adhatoda vasica* and Vasicine. The antioxidant potential of AVE was analyzed through *in vitro* assays. The protective effect of *Adhatoda vasica* extract (AVE) and vasicine were analyzed in TSE treated group through MTT assay and microscopic analysis.A dose dependent increase in reducing power of AVE was observed. Treatment of A549 & THP-1 cell lines with 1-2 µg/ml (AVE) & 0.01-0.02 µg/ml (Vasicine) respectively for 3 hrs maintained the cell viability. Approximately 50% cell death was observed at 2% & 5% TSE on 24 hrs exposure. Pre-treatment of cell lines with AVE & Vasicine (2µg/ml & 0.02 µg/ml) respectively could overcome the toxic effect of TSE. This study showed that AVE has a great potential in reducing the toxic effects of A549 &THP-1 cell lines.

**Key words:** Tobacco smoking, Adhatoda vasica, Vasicine, A549 & THP-1 cell lines.

## 1 INTRODUCTION

The rate of tobacco smoking is increasing worldwide, particularly in resource-poor countries (Slama *et al.,* 2008). The deleterious effects of smoking on health are severe and extensive, including impaired lung capacity, lung cancer, phlegm, cough, difficulty in breathing, bronchial trouble and vesicular emphysema (Hecht, 1999; Vainio *et al*., 2001).

*Adhatoda vasica* has been used to treat respiratory and other allergic conditions since years. It has been shown to treat many other diseased conditions like diabetes, ulcer etc. Vasicine is the main active ingredient of AV. It has also been proven to be a very good anti-inflammatory, anti allergic, and anti-ulcer etc. by various scientists (Rachana *et al*., 2011).

There are several medicinally plants proven to cure respiratory disorder to ameliorate this imbalance of oxidants and antioxidants in respiratory disorder. Many kinds of medicinal plants, which have been used as foods or medicinal purposes for a long time in Asia, are safe and may contain strong antioxidant activities.

This study is an attempt to evaluate the extent of toxicity caused by various concentrations of tobacco smoke and to check if, AV extract is able to overcome

Journal of Pharmaceutical Technology, Research and Management
Vol. 1
May 2013
pp. 109–117

the oxidative stress and cell death caused by the tobacco smoke in the human alveolar and macrophage cell lines (A549 & THP-1). The safe dose range of AVE for both the cell lines were determined by standard protocol. Further, the cells were pretreated with various concentrations of AVE (range 1-5µg/ml) and its active compound (Vasicine) (range 0.01-0.05µg/ml) and were then exposed to TSE (conc. 0.5-10%). The results obtained were compared with the respective controls to evaluate the modulation brought about with regards to cell viability in both the cell lines.



There are various approaches to determine that the biological system has undergone "oxidative stress" and because of tobacco smoking and has been tabulated in Table 1 (Bashan *et al.*, 2009).

**Table 1:** Parameters for assessing the "oxidative stress" in biological system

| Sl. No. | Ways of assessment | Parameters of choice |
|---|---|---|
| 1 | Pro-oxidant activity | NADPH oxidase, mitochondrial reactive oxygen species (ROS) |
| 2 | Antioxidant activity | Glutathione (GSH), Superoxide dismutase (SOD), Glutathione reductase (GR), Catalase, total antioxidant activity |
| 3 | Redox state of the cell | GSH/GSSG ratio |
| 4 | Oxidative damage | TBARS (thiobarbituric acid reactive substance), 4-HNE (4-hydroxynonenal), protein oxidation, DNA oxidation |

## 2 MATERIALS AND METHODS

### 2.1 Preparation of the ethanolic extract of Adhatoda vasica

*Adhatoda vasica* dried leaf powder was collect from Jeevan, herb, Madhay Pardesh. The powdered leaves of *A. vasica* were exhaustively extracted with 90% ethanol in a Soxhlet's apparatus. The ethanolic extracts of *A. vasica* (AVE) thus obtained, were collected and concentrated using rotary evaporator under reduced pressure at less than 40°C. Then the extracts were stored in aliquots at -80°C (stock) until used for further study.

### 2.2 Chemicals

The principle reagents used in this study and their sources are as follows. Ascorbic acid, dimethylsulfoxide (DMSO), penicillin, streptomycin, Nitro blue *tetrazolium* chloride and ABTS (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) were purchased from Sigma-Aldrich (St Louis, MO, USA).

Sodium carbonate anhydrous, sodium hydroxide, and riboflavin were sourced from CDH (New Delhi).Fetal bovine serum (FBS), RPMI-1640 and 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) from Himedia, Mumbai. All other reagents were of analytical grade. A549 & THP-1 cell line was obtained from the National Centre for Cell Science (NCCS) Pune, India.

## 2 3. Antioxidant Activity Measurement

### *ABTS (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) scavenging activity*

The ABTS scavenging activity of *A. vasica* was determined by the method of Brand-Williams *et al*. 1995. 7 mM ABTS solution and 2.4 mM potassium persulphate was added in equal volume and incubated for 12-16 hrs at room temperature in the dark. 1 ml of freshly prepared ABTS**+** solution was added in the resulting mixture. The samples of AVE and standard (BHT) were mixed individually with the resulting mixture in 1:1 ratio. After 10 mins of incubation, the absorbance was measured at 734 nm. The quenching or inhibition capacity of the extract for ABTS·+ and standard butylated hydroxyltoluene (BHT) was calculated. The percentage of scavenging capacity of the extract was calculated by the equation.

$$\text{ABTS radical scavenging activity (\%)} = [(\text{Abscontrol} - \text{Abssample})]/(\text{Abscontrol})] \times 100$$

where Abscontrol is the absorbance of ABTS radical + methanol; Abssample is the absorbance of ABTS radical + sample extract /standard.

## 2.4 Measurement of superoxide dismutase (SOD) activity

The scavenging activity of superoxide anion was determined by the riboflavin-NBT assay as adapted from Lai *et al*., 2008. To determine the SOD activity, The test sample (0.1 ml) at different extract concentrations were first mixed with 2.9 ml of 20 mM phosphate buffer (pH 7.2) containing 0.2 ml of 0.1 M EDTA and 0.1 ml of 1.5 mM NBT. After incubation at 37°C for 10 min, 0.05 ml of 1.2 mM riboflavin was added. The reaction mixture was illuminated with a 25 W light tube for 15 min in a foil-lined box. The microtiter plate reader was used to measure the absorbance at 560 nm. Deionized water instead of the extract was used as a control along with a sample blank which was evaluated by adding water instead of the riboflavin solution. The concentration of protein that provides 50% inhibition of the riboflavin mediated reduction of NBT, taken as SOD activity, was calculated.

### 2.5 Cell treatment & MTT assay

The human alveolar (A549) and macrophage (THP-1) cell lines were cultured in RPMI-1640 medium and on attaining 70 - 80% confluency, separately, they were seeded in 96-well plate at a density of $5x10^4$ cells (Dypbuky *et al*, 1994). The cells were treated with various percentage strength of tobacco smoke extract (TSE) for 24 hrs in order to determine its toxic dose that will lead to almost 50% cell death, thus generating enough cellular cytotoxicity. In the experiments where AVE and Vasicine were evaluated for its preventive effects, the cells were pre-exposed to different concentrations (1– 4 µg/ml) of AVE and Vasicine (0.01– 0.04 µg/ml) (prepared as per standard protocol) for 3 hrs. Cell viability was determined by MTT assay.



### 2.6 Microscopic analysis

Cells ($1x10^4$) were seeded onto six well plate and grown overnight in RPMI 1640 media with 10% FBS. Treatments of the cells were given according to three experimental sets **as** mentioned in MTT assay. After treatment, the cells were washed with PBS (pH 7.4, 20 mM) and then fixed with methanol. After fixing, the cells were again washed with PBS buffer and the plate was observed under inverted microscope at 40X without staining.

## 3 RESULTS

### 3.1 ABTS radical scavenging activity of AVE

In ABTS assay, the plant extract exhibited a dose dependent free radical scavenging activity against the stable radical (ABTS •) (Figure 1). The $IC_{50}$ value of the AVE required to scavenge 50% of ABTS • was 200 µg/ml.

### 3.2 Superoxide anion scavenging activity

As shown in the figure 2, AVE at a concentration of 0 – 100 µg/ml has shown a dose dependent free radical scavenging activity using NBT Reduction method. The 50% inhibition of NBT reduction by the ethanolic extract of AV has been found to be 55 µg/ml, as shown in Figure 2.

### 3.3 Effect of TSE on the viability of A549 and THP-1 cell lines

While evaluating the toxic effects of TSE, it was seen that 2% and 5% concentration can lead to almost 50% cell death in THP-1 & A549 cell lines respectively. As shown in Figure (3a & 3b) below, almost 50% cell viability

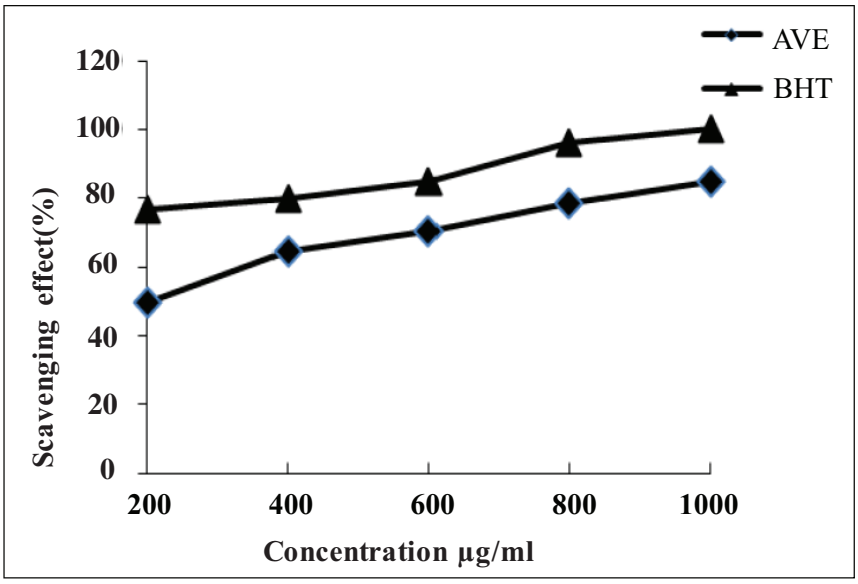


**Figure 1:** Free radical scavenging activity of AVE through ABTS assay

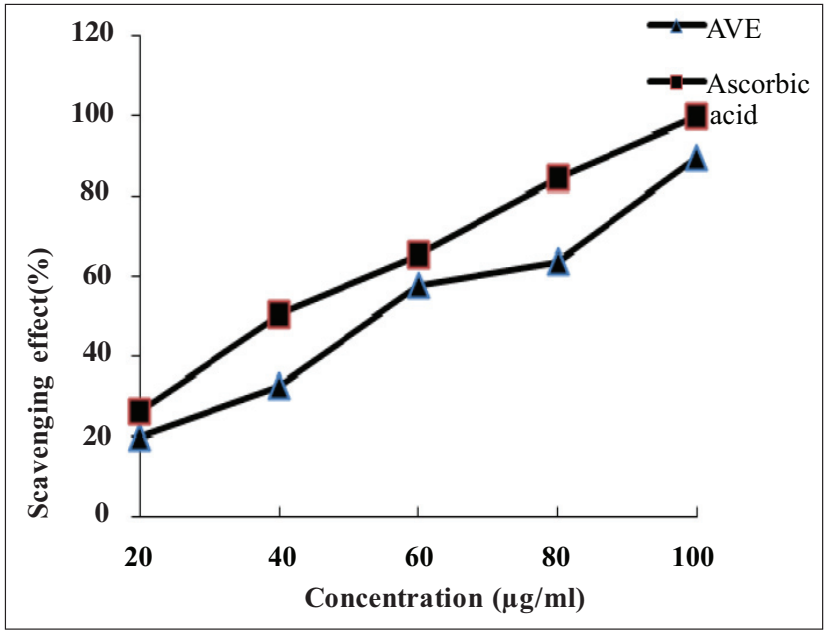


**Figure 2:** Superoxide radical scavenging activity of AVE and standard Ascorbic acid

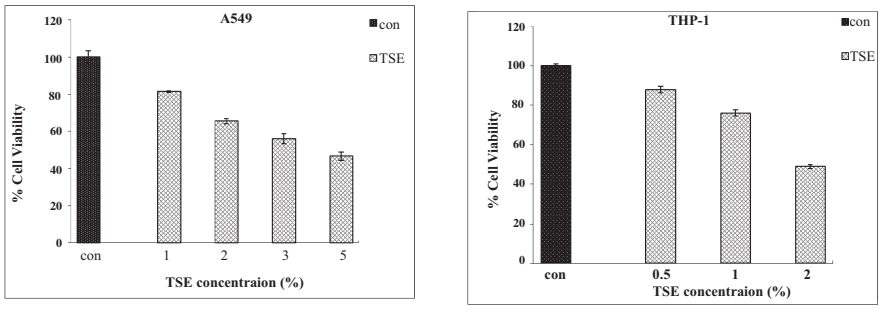


**Figure 3 (a & b):** Effect of treatment with various concentrations of tobacco smoke extract on A549 &THP-1 cell lines (5% and 2% TSE showed 50% cell death in A549 &THP-1 cell lines respectevely), Here con= control and TSE = Tobacco smoke extract.

was retained after exposing the cells to the above two TSE concentrations for 24 hrs.

### 3.4 Dose optimization for AVE and Vasicine

As shown in Figure 4(a & b) below, the cells were exposed to various concentrations of AVE for a time period of 3 hrs. MTT assay reveals that 1– 2 µg/ml and 0.01 – 0.02 µg/ml concentrations of AVE and Vasicine were in the safe range and they were not causing any harmful effects for the cells. Infect, the cell viability was found to be increased (118%, 120 % & 110%, 112%) &) with 2 µg/ml & 0.02 µg/ml concentrations of AVE & Vasicine in A549 and THP-1 respectively.

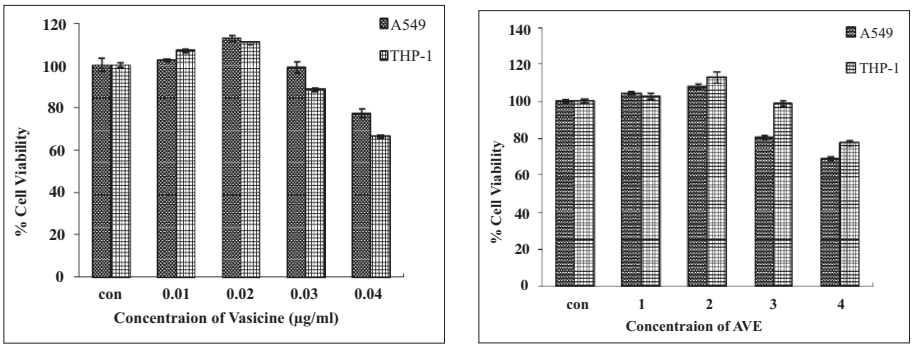


**Figure 4:** Assessment of viability of (A549 & THP-1) cells through MTT assay after treatment with various concentrations of AVE. Here con= control, AVE= Adhatoda vasica extract.

### 3.5 Preventive effect of AVE & Vasicine in TSE-induced toxicity

To check whether AVE can show beneficial effects on tobacco smoke extract treated cells, the cells were first exposed to 1– 2 µg/ml (AVE) and 0.01 – 0.02 µg/ml (Vasicine) followed by treatment with 2% & 5% TSE. As shown in figure 5 (a & b) below, the cells which were pre-exposed to AVE were more resistant to the toxic effects of TSE. 1 and 2 µg/ml AVE were found to be safe concentrations for the same.

### 3.6 Microscopic analysis

On treating the A549 cells with 5% TSE, significant change in cell shape, number, and size were observed under light microscope, as compared to control (Figure 6a) cell shrinkage was also observed. But, when the cells were pre-treated with AVE followed by 5% TSE, there was a significant increase in cell number as compared to TSE treated cells alone, but the cell size was almost similar to negative control (Figure 6e and 6f).

## 4 DISCUSSION

The polyphenols and polysaccharide that were found to be present in AVE has been shown to possess antioxidant, immunomodulatory and antiasthmatic properties (Rachana *et al.*, 2011). These phytoconstituents help fight against free radicals in the body.

As it has been reported to be a good antioxidant, it might be preventing the cell death caused by TSE by its nature. Similarly, its constituents like vasicine reported to modulate the antioxidant enzyme activities. There is a possibility that these mechanisms are involved in the protective role of AVE, and it is further required to specifically evaluate the mechanism of action of AVE and

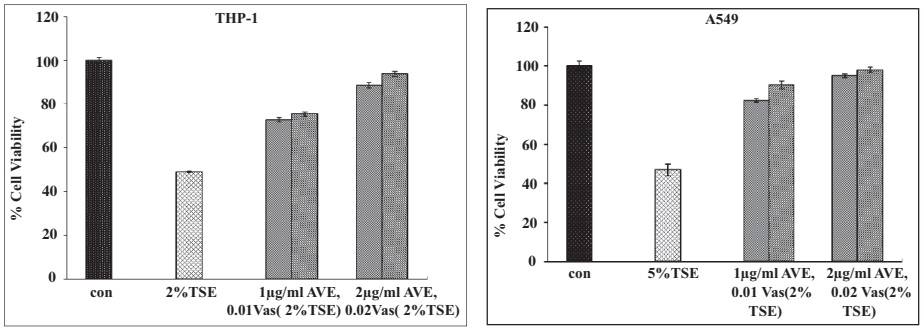


**Figure 5(a & b):** Protective effect of AVE & Vasicine on TSE treated A549 &THP-1 cells

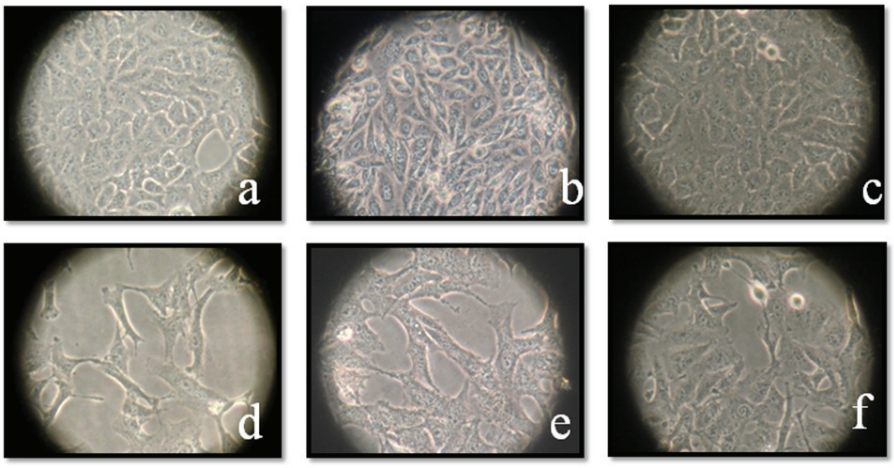


**Figure 6:** Microscopic analysis of A549 cells (a) control (untreated cells) (b) treated with 2 µg/ml AVE (c) treated with 0.02 µg/ml Vasicine (d) 5% TSE treated (e) 5%TSE treated cells which were pre-treated with 2 µg/ml AVE and (f) 5%TSE treated cells which were pre-treated with 0.02 µg/ml Vasicine.

the possible targets that could be ROS, oxidant enzymes and other biomarkers of oxidative stress.

Reports show that smoking generates free radicals in quantity enough to cause oxidative stress in several tissues including lungs.

## 5 CONCLUSION

This study reflects the protective role of AVE in preventing cell death of A549 & THP-1 cells caused by tobacco smoke extract. Further experimental studies are required to analyze the mechanism of action of the constituents of the AVE for their protective roles. The plant may thus help prevent or delay the onset of complications caused by smoking.

## REFERENCES

Bashan, N., Kovsan, J., Kachko, I., Ovadia, H. & Rudich, A. (2009). "Positive and negative regulation of insulin signaling by reactive oxygen and nitrogen species." *Physiol Rev*. Vol 89, pp 27-71. http://dx.doi.org/10.1152/physrev.00014.2008

Brand-Williams W, Cuvelier ME, Berset C. (1995). "Use of a radical method to evaluate antioxidant activity. *Lebensm-Wiss U-Technol* . Vol 28, pp 25-30. http://dx.doi.org/10.1016/S0023-6438(95)80008-5

Dypbukt J. M., Ankarcrona M., Burkitt M., Sjoholm A., Strom K., Orrenius S., Nicotera P. (1994). "Different prooxidant levels stimulate growth, trigger apoptosis, produce necrosis of insulin-secreting RINm5F Cells." *J Biol Chem*. Vol 269, pp 30553-30560.

Faizal P., Suresh S., Satheesh Kumar R., Augusti K. T. (2009).“A study on the hypoglycemic and hypolipidemic effects of an ayurvedic drug Rajanyamalakadi in diabetic patients”, *Indian J Clin Biochem.* Vol 24, pp 82-87. http://dx.doi.org/10.1007/s12291-009-0014-1

Hecht,S.S. (1999 ). ‘Tobacco smoke carcinogens and lung cancer”. *J .Natl. Cancer Inst*. 91: 1194-1210. http://dx.doi.org/10.1093/jnci/91.14.1194

Vainio, H., E. Weiderpass and P.Kleihues. (2001). “Smoking cessation in cancer prevention”. *Toxicol.* Vol 166, pp 47-52. http://dx.doi.org/10.1016/S0300-483X(01)00445-0

Lai, L.S. Chang, P.C. and Chang C.T. (2008). “Isolation and characterization of superoxide dismutase from wheat seedlings”. *J Agri and Food Chem,* Vol 56: pp 8121–8129. http://dx.doi.org/10.1021/jf800859f

Rachana, Basu S, Pant M, Kumar MP, Saluja S. (2011). “Review & Future Perspectives of Using Vasicine and Related Compounds”. *Indo-Global J Pharm Sci*. pp 85-98.

Slama K. (2008). “Global perspective on tobacco control. Part I. The global state of the tobacco epidemic”. *Int J Tuberc Lung Dis*. Vol. 12, pp 3–7.