

## Research Article

# CLINICAL USE OF ANDROGRAPHOLIDE AS A POTENTIAL DRUG AGAINST VOLE TUBERCULOSIS

H. K. Garg<sup>1</sup> and Ashish Shrivastava<sup>2\*</sup>

<sup>1</sup>Department of Zoology and Biotechnology, Sarojini Naidu Govt. Girls P. G. (Autonomous) College, Bhopal-462 016, India

<sup>2</sup>Department of Biotechnology, C.S.A. Govt. P. G. Nodal College, Sehore-466 001, India

**Article History:** Received 2<sup>nd</sup> July 2013; Revised form 20<sup>th</sup> July 2013; Accepted 14<sup>th</sup> August 2013; Published online 20<sup>th</sup> August 2013

---

## ABSTRACT

The current investigation deals with the potential anti-mycobacterial worth of andrographolide against *Mycobacterium microti*, commonly known as vole tuberculosis, employing different techniques viz. zone of inhibition, disc diffusion and turbidometric method. The bioactive compound, andrographolide, was isolated from the leaves of *Andrographis paniculata*. The MIC of andrographolide was found to be vacillating between 100 and 11.11µg/ml against the test organism. The results suggested andrographolide compound to be a potent antibiotic against *M. microti* and its inhibitory effect is on par with standard drugs like isoniazid and fluoroquinolones.

**Keywords:** Andrographolide, *Andrographis paniculata*, antibiotics, *Mycobacterium microti*, vole tuberculosis.

---

## INTRODUCTION

Tuberculosis (TB) in small wild mammals, namely field voles (*Microtus agrestis*), bank voles (*Clethrionomys glareolus*), wood mice (*Apodemus sylvaticus*) and shrews (*Sorex araneus*) was first reported by Wells and Oxon (1937) in Great Britain. Since then, a small number of studies have been carried out on vole tuberculosis and a meagre amount of information has been generated to depict the close relationship between *M. microti* isolates originating from different populations and the prevalence of this infection among wild rodent populations (Dickinson *et al.*, 1987)

Naturally occurring *Mycobacteria microti* (also known as vole bacillus) is a part of *Mycobacterium tuberculosis* complex. It is Gram-positive, non-motile and acid-fast rod,

Sensitive to isoniazid, ethambutol, rifampin, streptomycin and pyrazinamide and causes generalized tuberculosis in voles (Reed, 1957). There are many species of *Mycobacterium tuberculosis* complex such as *M. tuberculosis*, *M. bovis*, *M. caprae*, *M. africanum*, *M. microti* and *M. pinnipedi*. Though, these species show specific genetic homology, to some extent, yet there are prominent phenotypic differences in their relative pathogenicity for different mammalian species (World Health Organization, 1997). Field voles, bank voles, wood mice and shrews are susceptible to infection with *M. microti*.

*Andrographis paniculata* has been used in conventional *Siddha* and *Ayurvedic* systems of medication for multiple clinical applications since medieval times (Ahmad and Asmawi,

---

\*Corresponding author e-mail: akshrivastava333@gmail.com, Tel: +91 9301667033

1993). The therapeutic value of *Andrographis paniculata* is down to its mechanism of action, which is perhaps, by enzyme induction. Apart from its general use as an immune-stimulant, the plant extract exhibits anti-typhoid and anti-fungal activities too. According to a study conducted at Bastyr University, *Andrographis paniculata* possesses anti-hepatotoxic, anti-biotic, anti-malarial, anti-hepatitic, anti-thrombogenic, anti-inflammatory, anti-pyretic and anti-snake venom properties (Najib *et al.*, 1999).

The focal point of the present study is to endorse 'andrographolide' as an effective bioactive compound and its therapeutic application in treatment of vole tuberculosis.

## MATERIAL AND METHOD

### Plant extract

The leaves of *A. paniculata* were procured from nursery, dried in shade and, subsequently, in hot air oven for 30 minutes at low temperature and then pulverized into dust or powder form. The dried powder was then used for extraction with solvent ethanol (Misra *et al.*, 2011).

### Microorganisms

The strain, *M. microti*, used in the present study, was collected from MTCC IMTECH Chandigarh. *M. microti* was sub cultured and preserved in agar slants at 4° C and used for anti-mycobacterial study.

### Chemical analysis of Andrographolide

Thin layer chromatography (TLC) analysis was employed for the detection of andrographolide. A precoated plate of silica gel 60F254 (Merck) and mobile phase (chloroform: methanol: ethyl acetate - 8.0: 1.5: 1.0) were used. The ethanolic extracts fractionated by TLC were detected through UV radiation (Electronic UV Trans-illuminator: Rf of andrographolide = 0.58 (Sule *et al.*, 2010).

### Screening for antibacterial activity

The anti-mycobacterial activity of *Andrographolide* was scrutinized by using zone of inhibition, disc diffusion and turbidometric

method. Isoniazid and fluoroquinolones were taken as standard drugs for valid comparisons.

**In zone of inhibition method**,  $6 \times 10^4$  CFU of *M. microti* was spread on Middle brook 7H11 Agar media. Wells were dig on the plate with a hollow glass rod and were filled with equal concentrations of Andrographolide, Isoniazid & Fluoroquinolones in log 2 dilution. The experiment has a separate control for optimum growth of *M. microti*. After 21 days, radius of inhibition was measured in mm.

**In disc diffusion method**, sterile antibiotic discs (Himedia) were taken and filled up with 100, 33.33, 11.11, 3.77 and 1.23  $\mu\text{g/ml}$  of andrographolide using micropipette. At the same time, safety measures were taken to thwart the flow of the solvent extract from the outer surface of the disc. The discs were placed on the middle brook 7H11 agar plates on which the bacteria were inoculated, spread and incubated at 37° C for 21 days. The diameter of inhibition zone was measured in mm.

**In turbidometric method**, *M. microti* was added at the rate of  $10^8$  CFU/2 ml of 7H9 broth. Equal concentration of Andrographolide, Isoniazid and Fluoroquinolones were added in log 2 dilution. Again, the experiment has a separate control for optimal growth of *M. microti*. After 21 days O.D. was taken at 600 nm in spectrophotometer. Inhibitory activity of andrographolide was checked on the basis of optical density.

## RESULTS

Each experiment was done five times and the mean values of percentage inhibition were presented (Table 1). The results obtained from ZOI, DDA and TM were compared with standard antibiotics - isoniazid and fluoroquinolones. TM was found to be more effective assay for antibacterial study of *M. microti* than ZOI and DDM. The andrographolide achieved the greatest inhibitory feat ( $96.38 \pm 0.39$  %) at 100  $\mu\text{g/ml}$  concentration. The positive control for isoniazid and fluoroquinolones evinced  $98.89 \pm 0.45$  % and  $94.95 \pm 0.73$  % inhibition respectively at same concentration.

**Table-1.** Growth inhibition of *M. microti* by Andrographolide, Isoniazid and Fluoroquinolones.

	Concentration $\mu\text{g/ml}$	Andrographolide	Isoniazid	Fluroquinolones
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
100	Zone of inhibition	76.66 $\pm$ 6.41	89.80 $\pm$ 5.64	87.90 $\pm$ 4.34
	Disc diffution method	70.53 $\pm$ 3.90	69.62 $\pm$ 3.50	86.58 $\pm$ 4.56
	Turbidometric method	96.38 $\pm$ 0.39	98.89 $\pm$ 0.45	94.95 $\pm$ 0.73
33.33	Zone of inhibition	66.36 $\pm$ 4.97	75.70 $\pm$ 5.84	56.80 $\pm$ 4.34
	Disc diffution method	60.69 $\pm$ 2.10	63.89 $\pm$ 5.30	70.09 $\pm$ 1.95
	Turbidometric method	94.57 $\pm$ 0.08	95.71 $\pm$ 2.03	94.40 $\pm$ 1.95
11.11	Zone of inhibition	46.08 $\pm$ 4.37	34.20 $\pm$ 3.14	28.60 $\pm$ 3.82
	Disc diffution method	45.17 $\pm$ 2.30	26.80 $\pm$ 3.19	93.74 $\pm$ 3.81
	Turbidometric method	93.92 $\pm$ 0.84	93.80 $\pm$ 1.31	94.37 $\pm$ 1.11
3.77	Zone of inhibition	40.38 $\pm$ 4.04	28.20 $\pm$ 6.15	22.50 $\pm$ 4.34
	Disc diffution method	34.81 $\pm$ 3.70	23.00 $\pm$ 3.40	27.28 $\pm$ 4.36
	Turbidometric method	93.73 $\pm$ 0.86	84.93 $\pm$ 7.50	92.59 $\pm$ 2.11
1.23	Zone of inhibition	22.08 $\pm$ 2.80	15.00 $\pm$ 2.83	17.80 $\pm$ 4.85
	Disc diffution method	21.64 $\pm$ 4.50	17.80 $\pm$ 4.90	15.50 $\pm$ 1.90
	Turbidometric method	34.12 $\pm$ 5.02	27.40 $\pm$ 7.20	16.44 $\pm$ 9.56

Value are Mean of % inhibition with  $\pm$  SD (n = 5).

## DISCUSSION

Medicinal plants have been extensively used as pure compounds for alleviating diseases and disorders. However, only a few plant species have been thoroughly investigated for their medicinal properties. Gupta *et al.* (2010) found that the extracts of *A. indica*, *A. vasica*, *A. cepa*, *A. sativum* and *A. vera* exhibit anti-tuberculosis activity in L-J medium. Antony and James (2012) reported the extracts of *Alstonia scholaris* have 73.09% inhibition against a clinical strain, resistant to Streptomycin, Isoniazid, Rifampicin and Pyrazinamide.

## CONCLUSION

The increasing incidences of MDR and XDR-TB worldwide have prompted the urgent need to look for novel anti-tuberculosis drugs. In this wake, Andrographolide flares up a hope against the nuisance activities of different strains of *M. tuberculosis*.

## CONFLICT OF INTERESTS

The author declares that there is no conflict of interests associated with this article.

## ACKNOWLEDGEMENT

The authors are thankful to the head of the institutions for providing the necessary facilities.

## REFERENCES

- Ahmad, M. and Asmawi, M. Z. 1993. Some pharmacological effects of aqueous extract of *Andrographis paniculata* Nees. International Conference on the Use of Traditional Medicine and Other Natural Products in Health-care (Abstracts). University Sains, Malaysia, p. 122.
- Antony M. and James J., 2012. Antimycobacterial activity of plant extract *Alstonia scholaris*. *Int. J. Curr. Pharm. Res.*, 4(1): 40-42.
- Dickinson, J.M. and Mitchison, D.A. 1987. In vitro properties of rifapentine (MDL473) relevant to its use in intermittent chemotherapy of tuberculosis. *Tubercle*, 68:113-118.
- Gupta, R., Thakur, B., Singh, P., Singh, H.B., Sharma, V.D., Katoch, V.M. and Chauhan, S.V.S. 2010. Anti-tuberculosis activity of selected medicinal plants against multi-drug

- resistance *Mycobacterium tuberculosis* isolates. *Indian J. Med. Res.*, 131: 809-813.
- Misra, C.S., Pratyush, K., Sagadevan, L.D.M., James, J., Veetil, A.K.T. and Thankamani, V. 2011 A comparative study on phytochemical screening and antibacterial activity of roots of *Alstonia scholaris* with the roots, leaves and stem bark. *J. Res. Phytochem. Pharmacol.*, 1(2): 77-82.
- Najib, N.A., Rahman, N., Furuta, T., Kojima, S., Takane, K. and Ali, M.M. 1999. Antimalarial activity of extracts of Malaysian medicinal plants. *J. Ethnopharmacol.* 64: 249-254.
- Reed, G. 1957. Genus *Mycobacterium*: Species affecting warm-blooded animals except those causing leprosy, In: R.S. Breed, E.G.D. Murray and N.R. Smith (eds): *Bergey's Manual of Determinative Bacteriology*, 7<sup>th</sup> edition, The Williams & Wilkins Co, Baltimore.
- Sule, A., Ahmed, Q.U., Samah, O.A. and Omar, M.N. 2010. Screening for Antibacterial Activity of *Andrographis paniculata* used in Malaysian Folkloric Medicine: A Possible Alternative for the Treatment of Skin Infections. *Ethnobotanical Leaflets*, 14: 445-56, 2010
- Wells, A. Q., and D. M. Oxon. 1937. Tuberculosis in wild voles. *Lancet*, 1221.
- World Health Organization (WHO). 1997. Global tuberculosis control: Epidemiology, Strategy, Financing, pp. 6-33.