Chemopeventive Effects of Andographis Panniculata Extract in Vivo and in Vitro Models

Ramesh Chandra Agrawal [1*], Prachi Pandey [1], Neha Agrawa [1]

[1] Department of Research, Priyamvada Birla Cancer Research Institute, J.R.Birla Road, Satna 485005, Madhya Pradesh, India.

***Corresponding Author:** Ramesh Chandra Agrawal. Email: rcagrawal60@yahoo.com.

ABSTRACT

An Andrographis panniculata leaf extract was used against 7, 12 - dimethylbenz (a) anthracene (DMBA) induced papillomagenesis in Swiss albino mice was studied. Andrographis panniculata methanolic extracts were analyzed for Chemopreventive activity. Chemopreventive activity was evaluated using a two stage protocol consisting of initiation with a single topical application of a carcinogen (7, 12 - dimethylbenz (a) anthracene (DMBA) followed by a promoter (croton oil) twice a week. Tumor incidence, tumor burden and papillomas numbers reduction were observed, along with an increase in the average latent period for in mice treated topically with Andrographis panniculata extract compared to the control group treated with DMBA and croton oil alone. Andrographis panniculata antibacterial and antioxidant activities were also observed in the in vitro model revealing information about the anticancer, antibacterial and antioxidant activities of an Andrographis panniculata extract.

Key Words: Papilloma; DMBA; Croton oil; Antioxidant; Antibacteria

http://mo.qingres.com

OPEN ACCESS

DOI: 10.20900/mo.20160015

Received: May 12, 2016

Accepted: July 6, 2016

Published: August 25, 2016

1 INTRODUCTION

Cancer chemoprevention is defined as the use of specific natural and synthetic chemical agents to reverse or suppress carcinogenesis and prevent the development of invasive cancers. Recently there has been a growing awareness that dietary non-nutrient compounds can have important Chemopreventive effects. Considerable work examining the cancer Chemopreventive effects of such compounds in animal models has been undertaken. A number of common medicinal plants

have antioxidant properties and therefore may act as chemoprotector or radioprotectors. There is worldwide scientific interest in herbal based medicines to boost immune cells against cancer. By understanding the complex synergistic interaction of various constituents of anticancer herbs, herbal formulations can be designed to attack the cancerous cells without harming normal body cells.

Andrographis paniculata is known as "Kalmegh". It has been used for centuries in Asia to treat gastrointestinal (GI) tract and upper respiratory infections, fever, herpes, sore throat, and a variety of other chronic and infectious diseases. It appears in the Indian Pharmacopoeia and is prominent in at least 26 Ayurvedic formulas. Studies have confirmed that Andrographis, properly administered, has a broad range of pharmacological effects, some of them are beneficial [1]. The stem and leaves of the plant, used medicinally, contains a large number of chemical constituents, including lactones, diterpenoids, diterpene glycosides, flavonoids, and flavonoid glycosides. Controlled clinical trials report suggest its safe and effective for use in reducing symptoms of uncomplicated upper respiratory tract infections. A hydroalcoholic extract of Andrographis paniculata possesses antioxidant activity against oxidative alterations in the myocardium and confer significant cardio protection by facilitating normal cardiac function [2]. Compounds were isolated from chloroform and methanolic extract of Andrographis paniculata possess cytotoxic activity against cancer cell lines Hep G2, HCT-116 with MTT assay [3]. Antimicrobial activity against eleven bacterial strains by ethanol extract of the aerial part of Andrographis paniculata have been reported [4].

A purified extract and andrographolide have been reported to decrease blood glucose, triglyceride, and LDL levels when compared to controls. No changes were observed in the serum cholesterol or body weight of rats. Metformin has also shown similar effects in these parameters [5]. Andrographis paniculata Antiulcer activity was reported in cyst amine induced duodenal ulcer model in rats [6]. Andrographis paniculata antioxidant and hepatoprotective effect on acetaminophen (Paracetamol) induced hepatotoxicity in albino rats are also reported [7]. An andrographolide was reported to induce apoptosis in TD-47 human breast cancer cell line in a time and concentration-dependent manner by increase expression of p53, bax, and caspase-3 and decrease expression of bcl-2 determined by immune histochemical analysis [8]. Andrographis paniculata, dry leaf powder when fed orally to male albino rats, at a dose level of 20 mg per day for 60 days was reported to spermatogenesis cessation, cessation of degenerative changes in the seminiferous tubules, Leydig cells regression and regressive and/or degenerative changes in the epididymis, seminal vesicle, ventral prostate and coagulating gland [9]. An intraperitoneal injection of an ethanol extract of the aerial parts (25 g/kg body weight) in to mice poisoned with cobra venom delayed respiratory failure and death. These data suggest that extracts of the aerial parts do not modify the activities of the nicotinic receptors but produce significant muscarinic activity, which accounts for its antivenom effects [10]. Many of the conditions commonly treated with Andrographis paniculata in traditional medical systems are considered self-limiting, which requires purported benefit in cancer treatment.

2 MATERIALS AND METHODS

2.1 Chemicals

The croton oil, 7, 12 - Dimethylbenz (a) anthracene (DMBA), purchased from Sigma Chemicals Co. (St. Louis, MO. USA). Other chemicals were obtained from local firms and were of the highest purity. DMBA was dissolved in acetone at a concentration of 104 µg /100 µl . Croton oil was diluted in acetone to give a 1 % dilution.

2.2 Animals

Random bred male Swiss albino mice (7- 8 weeks old), weighing 24 ± 2 gm were used. These animals were housed in polypropylene cages at temperatures of 24 ± 3°C.animals and animals were provided with standard mice feed and tap water ad libitum.

2.3 Preparation of Andrographis panniculata extract

Plant material (Andrographis panniculata) was collected locally and identified and the specimen was authentified by the Department of Botany, Safia College, Bhopal (MP), India. Bark was washed, air dried, powdered and extracted separately using 50 % methanol and a separating funnel. Extract thus obtained were vacuum evaporated into powder. These extract was again dissolved in DDW immediately prior topical application.

2.4 Experimental design for Skin Carcinogenesis

The dorsal skin on the animal's back was shaved 1 day before the experiment commenced. Only animals in the hair cycle resting phase were chosen.

Two stage protocol initiated by a single topical application of a carcinogen (7, 12 - dimethylbenz (a) anthracene (DMBA) and then followed by a promoter (croton oil) twice in a week were employed per Berenblum [11] as standardized by Agrawal *et al.*, [12]) used to induce tumours. Animals were randomly allocated into 7 groups of comprising six mice each. The treatment was provided topically to the shaved area

2.4.1 Treatment Groups

Group 1 (Untreated control): No treatment

Group 2 (Vehicle control): Twice a week administration of 100 µl acetone up to 16 weeks

Group 3 (DMBA Alone): - Single administration of 104 µg DMBA dissolved in 100 µl acetone.

Group 4 (Croton Oil Alone): -Twice a week application to skin of 1 % Croton oil up to 16 week.

Group 5 (Andrographis panniculata Extract Alone): - Twice a week application to skin at the dose of 500 mg/kg b. wt up to 16 week.

Group 6 (DMBA + Croton Oil): - Single application to skin of 104 µg DMBA in 100 µl acetone afterwards 1 % croton oil was applied on skin twice a week up to 16 week.

Group 7 (DMBA + Andrographis panniculata Extract + Croton Oil): - Single application to skin of 104 µg DMBA in 100 µl acetone afterwards the 100 µl dose of Andrographis panniculata extract at the dose of 500 mg/kg b. wt. was given one hour before the each application of 1 % croton oil twice a week up to 16 weeks.

All animals groups were observation for gross and microscopic skin changes weekly during the 16 weeks of experimentation period. All mice were weighed and examined for skin papillomas and results were recorded. The following parameters were considered

2.4.2 Tumor study

Body weight: Mean body weight changes was measured weekly.

Tumor incidence: The number of mice at least one tumor expressed as percent incidence.

Cumulative number of papillomas: Total number of tumors bearing mice.

Tumor yield: The average number of papillomas per mouse.

Tumor burden: The average number of tumors per tumor bearing mouse.

Average latent period: The lag between the application of the promoting agent and the appearance of 50 % tumors was determined. The average latent period was calculated by multiplying the number of tumors appearing each week by the time in weeks after the application of the promoting agent and dividing the sum by total number of tumors.

Average latent period = $\Sigma fx/n$

where f is the number of tumors appearing in each weeks, x is the numbers of weeks and n is the total number of tumors.

2.4.3 Anti-bacterial activities

The antibacterial activity of a hydro-methanolic extract from leaves of Andrographis panniculata was investigated using a Disk diffusion method given by Kerby-Bauer.

Bacterial strain:Following gram negative and gram positive bacterial strain i.e. and E.coli, Klabsella, Staphylococcus and Pseudomonas were used for the Antibacterial activities which were received from stock culture of our laboratory.

Media: Nutrient agar broth media were used for the antibacterial activities. Nutrient broth is prepared i.e. 1.3g in 100ml of double distilled water, poured in 6 different test-tubes and added 4 bacterial strain in each test-tube. Nutrient Agar media prepared poured in Petriplates after solidifying swab the bacterial cultures on the plates and allowed for incubation at 370C for 24 hrs.

Concentration: Four different concentrations of crude extract were prepared (100 %, 75 %, 50 % and 25 %). 100 % = 1g crude extract in 1ml of double distilled water freshly prepared afterward serial dilution prepared 75 % = 75mg in 1ml, 50 % = 50mg in 1ml and 25 % = 25mg in 1ml.

Study parameter: Measurement of Zone of Inhibition (In mm)

2.4.4 Anti-oxidant activities

Anti-oxidant activities of Gymnema sylvestre extract (10-100 µg/ml) were determined according De-oxyribose method (Fenton reaction) of Halliwell and Aruoma [13]. The hydroxyl radical attacked to de-oxyribose and initiated a series of reaction that eventually resulted in the formation of thiobarbeturic acid reaction substances (TBARS). Ascorbic acid= 1mg in 1ml,

Formula: % Inhibition = Abs. 532nm control-Abs. 532nm test Abs./532nm control x 100

3 RESULTS

The study's findings depicted in Tables I. Animals of Group- VI (control) in which a single topical application of DMBA, followed by croton oil produced skin papillomas, which began appearing in the started 5th week. Papilloma incidence in DMBA/ croton oil treated mice (carcinogen control) reached 100 % by the endof the experiment (16 weeks).

Table 1. Showing Cumulative No. of Papilloma in the animals treated with Andrographis panniculata extract

Groups	Dose	Time of 1st appearance of Papilloma	Cumulative No. of Papilloma	Tumour yield	Tumour incidence
Vehicle alone	100µl/animal	–	–	–	–
DMBA alone	104µg/animal	–	–	–	–
Croton Oil alone	1 % per animal	–	–	–	–
Andrographis Extract alone	mg/kg per animal	–	–	–	–
DMBA + CO (Control)	104µg + 1 % per animal	58th Day	47	47/6 (7.8)	6/6 (100 %)
DMBA+CO+ Andrographis Extract.	104µg + 1 % + 400mg/animal	61th Day	11	11/5 (2..2)	5/6 (80 %)

* Denotes a statistical significance compared with DMBA+ croton oil group at $p < 0.05$

In a skin papilloma model, instances of significant tumour prevention observed in the Andrographis panniculata extract treated experimental groups (80 % in group VII) compared to the carcinogen control (100 %) group. Papillomas cumulative number was also reduced in the Andrographis panniculata leaves extract treated experimental groups (11 in group VII) compared to carcinogen control group. Tumor burdens and tumor yields were also decreased (2.2) compared to DMBA treated control (7.8) group.

Andrographis panniculata leaves at the different concentration(25 %, 50 %, 75 % and 100% exhibited antibacterial activity against and E. coli, Klabsella, Staphylococcus and Psuedomonas (Table 2).

Table 2. Antibacterial activity of Andrographis panniculata against bacterial strains

Microorganisms	Extract Concentration as % [zone of inhibition(mm)]			
	25	50	75	100
Klebsialla	14	15	16	18
E. Coli	12	14	16	18
Staphylococcus	14	15	17	19
Psuedomonas	13	14	16	16

Andrographis panniculata leaves in vitro antioxidant activity was tested in various concentrations against ascorbic acid as the standard. Percentage of TBARS was calculated for both ascorbic acid and Andrographis panniculata extract, with the help of formula, for a comparative study. TBARS percentage was calculated and plotted in the graph in different concentration (Table 3).

Table 3. In vitro antioxidant activity of 50% methanolic Andrographis panniculata extracts vs Ascorbic acid (standard)

Sr. No.	Ascorbic acid (Concentration µg)	% TBARS inhibition	Concentration of Andrographis panniculata (µg)	% TBARS inhibition
1	50	25	10	8.2
2	100	73	20	52.7
3	150	165	30	24.02
4	200	96	40	36.38
5	250	55	50	46.6
6	300	144	60	46.3
7	350	145	70	55.2
8	400	171	80	66.2
9	450	409	90	73.3
10	500	270	100	104.5

IC50 Andrographis panniculata value is between 50-60 µg

4 DISCUSSION AND CONCLUSION

Chemoprevention is currently an important strategy for controlling of cancer induction. There is a need to explore medicinal plants or other natural agents that may be work as chemopreventive agents. The current study demonstrates a chemopreventive potential for andrographis panniculata extract for DMBA-induced skin tumorigensis in male Swiss albino mice. Skin carcinogenesis model in experimental animals has been found to be a useful when for investigating the chemopreventors influences both mechanistically and operationally [15]. The present study demonstrats that a topical application of andrographis panniculata extract (500 mg/kg body weight) at the pre promotion phase shows a significant reduction in tumor incidence, tumor burden, tumor weight, tumor size, and cumulative number of papillomas in andrographis treated groups relative to the carcinogen treated control. No androgrphis panniculata anticarcinogenic activity has not been well documented. Evidence has accumulated suggesting that this may be due to a reactive oxygen species. which play an important role in tumor initiation/promotion by enhancing or facilitating the metabolic activation and/or initiating effects of carcinogens [19]. The plant extract may have inhibited the DMBA metabolism to its active form, delayed the carcinogenesis promotion phase or down regulated reactive oxygen species formation. [16-18]. There are few reports on the cytotoxic and antiproliferative effects of Andrographis. paniculata up on in vitro cell lines [8, 13, 14]. Andrographolide, a Andrographis panniculata compound was also reportedly induced apoptosis in a TD-47 human breast cancer cell line in a time and concentration-dependent manner. It also increased expression of p53, bax and caspase-3 and decreased bcl-

2 expression as shown by immunohistochemical analysis was observed [8]. The anticarcinogenic activity of andrographis panniculata in skin papilloma model in Swiss albino mice was observed. The antibacterial and antioxidant effect of andrographis panniculata was also observed an in vitro model. The present study suggests that Andrographis . panniculata may be an important drug in traditional medicine to treat various ailments.

ACKNOWLEDGEMENTS

The authors are thank the M. P. Council of Science and Technology, Bhopal for its financial assistance for the above work.

REFERENCES

1. Sandberg F. Andrographidis herba Chuanxinlian: A review. Gothenburg, Sweden: Swedish Herbal Institute. Available from the American Botanical Council (USA). 1994.
2. Ojha SK, Bharti S, Joshi S, Kumari S, Arya DS. Protective effect of hydroalcoholic extract of Andrographis paniculata on ischaemia-reperfusion induced myocardial injury in rats. Indian J Med Res. 2012; 135(3): 414-421.
3. Aditya VSPK, Kumar N L, Mokkapati A. Evaluation of in vitro cytotoxicity of Andrographis paniculata, Duranta serratifolia and Albizzia lebbeck whole plant extracts by MTT assay against MCF-7 and HT-29 cell lines. Cur Res Microbiol Biotechnol. 2014; 2(2): 351-353.
4. Mishra US, Mishra A, Kumari R, Murthy PN, Naik BS. Antibacterial activity of ethanol extract of Andrographis paniculata. Indian J Pharm Sci. 2009; 71(4): 436-438.
5. Nugroho AE, Mohamad A, Endang L. Antidiabetic and antihiperlipidemic effect of Andrographis paniculata (Burm. f.) Nees and andrographolide in high-fructose-fat-fed rats. Indian J Pharmacol. 2012; 44(3): 377-381.
6. Saranya P, Geetha A. Antiulcer activity of Andrographis paniculata (Burm.f.) Wall against cysteamine-induced duodenal ulcer in rats. 2011; 49(7): 525-33.
7. Maleeka Begum SF, Santhana Kumar J, Swathi Balakrishnan V. Antioxidant and hepatoproective effect of Andrographis paniculata against acetaminophen (paracetamol) induced hepatotoxicity in Albino rats. Int J Cur Res. 2011; 3(12): 64-68.
8. Harjotaruno S, Widyawaruyanti A, Noor CZ. Apoptosis inducing effect of andrographolide on TD-47 human breast cancer cell line. African J Tradit Complimen Alt Med. 2007; 4(3): 345-351.
9. Akbarsha MA, Murugaian P. Aspects of the male reproductive toxicity/male antifertility property of andrographolide in albino rats: effect on the testis and the cauda epididymidal spermatozoa. Phytother Res. 2000; 14(6): 432-435.
10. Meenatchisundaram S, Parameswari G, Subbraj T, Suganya T, Michael A. Medicinal and Pharmacological Activities of Andrographis paniculata–Review. Ethnobotanical Leaflets. 2009; 13: 55-58.
11. Berenblum I, Shubik P. A new quantitative approach to the study of stages of chemical carcinogenesis in the mouse skin. Br J Cancer. 1947; 1(4): 383-391.
12. Agrawal RC, Jain R, Wasim R, Ovais M. Anticarcinogenic effects of solanum lycopersicum fruit extract on swiss albino and C57 BL mice. Asian Pacific J Cancer Prev. 2009; 10(3): 379-382.
13. Rajagopal S, Kumar RA, Deevi DS, Satyanarayana C, Rajagopalan R. Andrographolide, a potential cancer therapeutic agent isolated from andrographis paniculata. J Exp Ther Oncol. 2003; 3(3): 147-158.
14. Geethangali M, Rao YK, Fang SH, Tzeng YM. Cytotoxic constituents from Andrographis paniculata induce cell cycle arrest in jurkat cells. Phytother Res. 2008; 22(10): 1336-1341.
15. Morse EC, Stoner G. Cancer chemoprevention — principles and prospects. Carcinogenesis. 1996; 14(9): 1737-1746.
16. Kausar H, Bhasin G, Zargar MA, Ather M. Palm oil alleviates 12-O tetradecanoyl–phorbol-13 acetate-induced tumor promotion response in murine skin. Cancer Lett. 2003; 192: 151-160.
17. Sancheti G, Jandal A, Kumari R, Goyal PK. Chemopreventive action of Emblica officinalis on skin carcinogenesis in mice. Asian Pac J Cancer Prev. 2005; 6(2): 197-201.
18. Kumar M, Soni KA, Shukla S, Kumar A. Chemopreventive potential of Tribulus terrestis against 7,12 dimethylbenz (a)anthracene induced skin papillomagenesis in mice. Asian Pac J Cancer Prev. 2006; 7(2): 289-294.
19. Ather M. Oxidative stress and experimental carcinogenesis. Indian J Exp Biol. 2002; 40(6): 656-667.