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

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ABSTRACT

Andrographis panniculata leaves extract against 7, 12 - dimethylbenz (a) anthracene (DMBA) induced papillomagenesis in Swiss albino mice was studied. The methanolic extracts of andrographis panniculata were analyzed for Chemopreventive activity. Chemopreventive activity was evaluated by 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) two times in a week were employed. A significant reduction in tumor incidence, tumor burden and cumulative number of papillomas was observed, along with a significant increase in an average latent period in mice treated topically with andrographis panniculata extract as compared to the control group that was treated with DMBA and croton oil alone. The antibacterial and antioxidant activities of andrographis panniculata were also observed in vitro model. The above studies revealed information about the anticancer, antibacterial and antioxidant activity of andrographis panniculata extract. Therefore, the present study is immensely important in future drug development programs for the cancer treatment.

Key words: Papilloma; DMBA; croton oil; antioxidant; antibacterial

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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. There has been a growing awareness in recent that dietary non-nutrient compounds can have important effects as Chemopreventive agents, and considerable work on the cancer Chemopreventive effects of such compounds in animal models has been undertaken. A number of common medicinal plants have good antioxidant properties and therefore may act as chemoprotector and radio protector. Scientists all over the world are concentrating on the herbal medicines to boost immune cells of the body against cancer. By understanding the complex synergistic interaction of various constituents of anticancer herbs, the herbal formulations can be designed to attack the cancerous cells without harming normal cells of the body.

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 is found in the Indian Pharmacopoeia and is the prominent in at least 26 Ayurvedic formulas. Studies have confirmed that Andrographis, properly administered, has a surprisingly broad range of pharmacological effects, some of which are extremely beneficial [1]. The aerial part of the plant, used medicinally, contains a large number of chemical constituents, mainly including lactones, diterpenoids, diterpene glycosides, flavonoids, and flavonoid glycosides. The reports of controlled clinical trials suggest its safe and effective use for reducing symptoms of uncomplicated upper respiratory tract infections. Hydro alcoholic extract of Andrographis paniculata possesses antioxidant activity against oxidative alterations in myocardium and confer significant cardio protective activity by helping in retaining the cardiac function in a normal manner [2]. The compounds were isolated from chloroform and methanolic extract of Andrographis paniculata possessed cytotoxic activity against cancer cell lines Hep G2,HCT-116 with MTT assay [3]. Ethanol extract of the aerial part of Andrographis paniculata was reported for antimicrobial activity against eleven bacterial strains [4].

The purified extract and andrographolide were reported to decrease the levels of blood glucose, triglyceride, and LDL compared to controls. However, no changes were observed in serum cholesterol and body weight of rats. Metformin also showed similar effects on these parameters [5]. Antiulcer activity of Andrographis paniculata was reported by cyst amine induced duodenal ulcer model in rats [6]. The antioxidant and hepatoprotective effect of Andrographis paniculata on Acetaminophen (Paracetamol) induced hepatotoxicity in albino rats were also reported [7]. The andrographolide was reported to induce apoptosis in TD-47 human breast cancer cell line in a time and concentration-dependent manner by increasing the expression of p53, bax, and caspase-3 and decreasing expression of bcl-2 determined by immune histochemical analysis [8]. Dry leaf powder of Andrographis paniculata, was reported that when fed orally to male albino rats, at a dose level of 20 mg per day for 60 days, it resulted in cessation of spermatogenesis, degenerative changes in the seminiferous tubules, regression of Leydig cells and regressive and/or degenerative changes in the epididymis, seminal vesicle, ventral prostate and coagulating gland [9]. The intraperitoneal injection of an ethanol extract of the aerial parts (25 g/kg body weight) to mice poisoned with cobra venom markedly delayed the occurrence of 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]. Since many of the disease conditions commonly treated with Andrographis paniculata in traditional medical systems were considered self-limiting, a critical evaluation was required for its purported benefits in cancer treatment.

MATERIALS AND METHODS

1. Chemicals

7, 12 - Dimethylbenz (a) anthracene (DMBA), croton oil from Sigma Chemicals Co. (St. Louis, MO. USA). The 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 and croton oil was diluted in acetone to 1 % dilution.

2. Animals

Random bred male Swiss albino mice (7- 8 weeks old), weighing 24 ± 2 gm were used for the experiments. These animals were housed in polypropylene cages in the animal house at temperatures of 24 ± 3^0C. The animals were provided with standard mice feed and tap water ad libitum.

3. Preparation of Andrographis panniculata Extract

Plant material (Andrographis panniculata) was collected locally and identified and the specimen was authenticated at Department of Botany, Safia College, Bhopal(MP), India. Bark was washed, air dried, powdered and extracted separately, with 50 % methanol, using separating funnel.

Extracts thus obtained were vacuum evaporated to make them into powder form. These extracts were again dissolved in DDW just before topical application.

4. Experimental design for Skin Carcinogenesis

The dorsal skin on the back area of the animals was shaven 1 day before the commencement of the experiment and only those animals in the resting phase of the hair cycle were chosen for the study. For induction of tumors 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) two times in a week were employed as per method of Berenblum [11] and standardized by Agrawal et al [12]. The animals were randomly allocated into 7 groups comprising six mice each. The treatment was provided topically on shaved area.

4.1 Treatment Groups

Group 1 (Untreated control): No treatment

Group 2 (Vehicle control): 100 µl acetone 2 times /week up to 16 weeks

Group 3 (DMBA Alone): - 104 µg DMBA was dissolved in 100 µl acetone and single application was given.

Group 4 (Croton Oil Alone): - 1 % Croton oil was applied on skin 2 times a week up to 16 week.

Group 5 (Andrographis panniculata Extract alone):

- was applied at the dose of 500 mg/kg b. wt on skin 2 times a week up to 16 week.

Group 6 (DMBA + Croton Oil): - 104 µg DMBA was dissolved in 100 µl acetone and single application was given afterwards 1 % croton oil was applied on skin 2 times a week up to 16 week.

Group 7 (DMBA + Andrographis panniculata Extract + Croton Oil): - 104 µg DMBA was dissolved in 100 µl acetone and single application was given afterwards the 100 µl dose of andrographis panniculata extract at the dose of 500 mg/kg b. wt. was given one hour before each application of 1% croton oil 2 times a week up to 16 weeks.

The animals of all groups were kept under observation for gross and microscopic changes in skin. During the period of 16 weeks of experimentation, mice of all groups were weighed carefully and examined once a week for skin papillomas and they were recorded. The following parameters were taken into consideration.

4.2 Tumor study:

Body weight: Change in mean body weight was measured weekly.

Tumor incidence: The number of mice carrying 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.

4.3 Anti-bacterial activities

Antibacterial activities of hydro-methanolic extract from leaves of andrographis panniculata were investigated using the Disk diffusion method given by Kerby-Bauer Disk Diffusion Susceptibility test.

Bacterial strain:

Following gram negative and gram positive bacterial strain i.e. 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 was prepared i.e. 1.3 g in 100 ml 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 Petri plates after solidifying swab the bacterial cultures on the plates and allowed for incubation at 37 ^{0}C 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 % = 75 mg in 1ml, 50 % = 50mg in 1ml and 25 % = 25mg in 1ml.

Study parameter

Measurement of Zone of Inhibition (In mm)

4.4 Anti-oxidant activities

Anti-oxidant activities of andrographis panniculata 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 = 1 mg in 1 ml.

Formula:

% T Bars Inhibition = Absorbance at 532 nm control - Abs. 532 nm test Abs./ 532 nm control x 100

RESULTS

The findings of the present tumor study was depicted in Table 1. Animals of Group- VI (control) in which a single topical application of DMBA, followed by croton oil produced skin papillomas, which started to appear from the 5th week onwards. The incidence in DMBA/croton oil treated mice (carcinogen control) reached 100 % by the termination of the experiment (i.e. 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	Tumor yield	Tumor 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 Ext.	104µg + 1% + 500mg/animal	61st Day	11	11/5* (2..2	5/6 (80 %)

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

In the skin papilloma model, significant prevention of tumor incidences was observed in the andrographis panniculata extract treated experimental groups (80 % in group VII) compared to carcinogen control (100 %) group. The cumulative number of papillomas was also reduced in the andrographis panniculata leaves extract treated experimental groups (11 in group VII) compared to carcinogen control group. The tumor burden and tumor yield were also decreased (2.2) as compared to DMBA treated control (7.8) group.

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

Table 2 Antibacterial activity of Andrographis panniculata against Different Bacterial Strains

Name of microorganisms	% Concentration of Extract [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

The result shows dose dependent inhibition of bacterial colonies.

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

Table 3 In vitro Antioxidant Cctivity of 50 % Methanolic Andrographis Panniculata Extracts Vs Ascorbic Acid

Sr. No.	Concentration of ascorbic acid (µg)	%TBARS inhibition	Concentration of Andrographis panniculata (µg)	% TBARS inhibition
1	50	25	10	8.2
2	100	73	20	22.7
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 Value of andrographis panniculata extract is between 50-60 µg.

DISCUSSION AND CONCLUSION

Chemoprevention is currently an important strategy for controlling the process of cancer induction. Therefore, there is a need to explore medicinal plants or other natural agents that can work as chemopreventive agents. The current study demonstrates the chemopreventive potential of andrographis panniculata extract on DMBA-induced skin tumorigensis in male Swiss albino mice. The skin carcinogenesis model in experimental animals has been found to be a very useful system for investigating the influence of dietary chemopreventors both mechanistically and operationally [15]. The present study demonstrated that topical application of the andrographis panniculata extract (500 mg/kg body weight) at the pre promotion phase showed 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 mechanism of anticarcinogenic activity of androgrphis panniculata extract has been documented. However, evidence has been accumulated to suggest that this is perhaps due to 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 metabolism of DMBA to its active form, delayed the promotion phase of carcinogenesis, or down regulated reactive oxygen species formation [16-18]. There are few reports about the cytotoxic and antiproliferative effects of Andrographis paniculata in in vitro cell lines [8, 13, 14]. Andrographolide, a compound from Andrographis panniculata was also reported to induce apoptosis in TD-47 human breast cancer cell line in a time and concentration-dependent manner by increasing expression of p53, bax and caspase-3 and decreasing expression of bcl-2 determined by immuno histochemical analysis [8]. We observed the anticarcinogenic activity of andrographis panniculata in skin papilloma model in Swiss albino mice. The antibacterial and antioxidant effect of andrographis panniculata was also observed in vitro model. The present study is immensely important because andrographis panniculata is an important drug in traditional medicine to treat various ailments.

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Declaration of interests

The authors declare no conflict of interests.

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