

Modulation of carcinogenicity by *Andrographis paniculata* extract

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Abstract- In the present investigations, the anticarcinogenic activity of *Andrographis paniculata* leaves and whole plant extracts was evaluated using two stage protocol in skin papilloma model in *Swiss albino* mice. It is a widely used medicinal plant and used in various indigenous systems of medicine. The prevention of papillomas in DMBA + *Andrographis paniculata* leaves and whole Plant extract + croton oil treated group was observed in The mice which was initiated by DMBA followed by croton oil. The different parameters like tumor incidence, cumulative number of papillomas, tumor yield and tumor burden were measured which showed decreased in *Andrographis* extract treated groups. Whole plant extract of *Andrographis Paniculata* showed cytotoxic activity in different concentration starting from 10µg/ml and leaf extract of the plant showed in the concentrate from 250µg/ml after 24 hour. The glutathione level was also measured in blood and liver tissues of treated mice which showed increased level in the animals which received the treatment of *Andrographis* extract along with DMBA + Croton Oil. The results showed that *Andrographis* extracts have cytotoxic and anticarcinogenic potential in the test systems used.

INTRODUCTION

Medicinal plants are an important flora which are widely distributed in India. Plant-based medicines still play an important role in the primary healthcare of 80% of the world's population in both underdeveloped and developed countries (De S., 2012).

One of the most dreaded diseases of the recent Century is Cancer that is spreading further with increasing in 21st century. Cancer is an uncontrolled growth of cells resulting in lack of differentiation and ability to invade local tissues and metastasis which are proliferate individually throughout the body. During metastasis, cancer cells enter the blood stream and are carried forward to distant parts of the body where they form other similar growths (Jemal et al., 2008). Cancer involves changes or mutations in the cell genome. These changes (DNA mutations) produce disrupt the delicate cellular balance between cell division and quiescence, resulting in cells that keep dividing to form cancers. Cancer is responsible for several deaths worldwide more than AIDS, tuberculosis, and malaria together (Sener SF., 2005).

Nature has provided human a variety of useful sources mainly plants for discovery and development of drugs against dreadful diseases (Joselin and Jeeva., 2014). India has been identified as a major resourceful area in the traditional and alternative medicines globally. *Andrographis paniculata* is commonly known as the “king of bitters” because of its taste. It is a herbaceous plant belonging to the Acanthaceae family and is found throughout tropical and subtropical Asia, Southeast Asia (R. A. Kumar et al., 2004); and Asian countries like India, Sri Lanka, Pakistan, Java, Malaysia and Indonesia (Shahid A., 2011; Kabeeruddin M.,1937). In India, *A. paniculata* is known as “Kalmegh” (R. A. Kumar et al., 2004). The genus *Andrographis* consists of 28 species of small annual herbs essentially distributed in tropical Asia. *A. paniculata* is the most popular in medicinal plants (Chopra RN et al., 1956) and ranks seventeenth position among their thirty two prioritized medicinal

plants (Anonymous., 2007). *A. paniculata* is an important constituent of at least 26 Ayurvedic formulas in Indian pharmacopoeia (Deng WL.,1978). The medicinal value of *A. paniculata* plant is due to the presence of active ingredients viz andrographolide and neoandrographolide which are derivatives of diterpenoids present in leaves (Mohammad Abu Bin Nyeem., 2017). This plant contains important bioactive compounds steroids, phenols, terpenoids, alkaloids, saponins, flavonoids and xanthenes that showed an important pharmacological activities such as antidiabetes, antidiarrheal, antibacterial, cardiovascular benefits, anti-inflammatory and hepato protective benefits (Jarukamjorn et al., 2010; Mukherjee et al., 2006; Mohammad Abu Bin Nyeem., 2017). Since cancer treatment in traditional medical systems is being considered, therefore we have planned to carry out this study to evaluate the anticarcinogenic effect of *Andrographis* extract in experimental animals by using *Skin Papilloma and cell toxicity studies using Hela Cells line in Vitro of Andrographis paniculata* leaves and whole Plant extracts.

Materials and Methods

Chemicals

7, 12 - Dimethylbenz (a) anthracene (DMBA), croton oil, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma Chemicals Co. ,St. Louis, MO. USA. The other chemicals were obtained from local firms and were of the highest purity.

Animals

The experimental study was conducted on random bred, 6-7 weeks old and 24- 28 gm body weight of male *Swiss albino* mice. Animals were maintained under controlled conditions of temperature ($24 \pm 3^{\circ}\text{C}$) and light (Light: dark, 10 hrs: 14 hrs.). The animals were provided with standard mice feed and tap water *ad libitum*. The experiment was approved by Institutional animal ethic committee before conduction of the experiment.

Preparation of *Andrographis paniculata* Leaves and Whole Plant Extract

Plant material of *Andrographis paniculata* was collected and the specimen was authenticated by the botanist of Deendayal Research Institute, Chitrakoot, Satna, Madhya Pradesh (India). The non-infected leaves and whole Plant was washed, air dried, powdered and extracted separately using 50 % methanol in a separating funnel. Extract thus obtained were vacuum evaporated into powder. These extract was again dissolved in DDW immediately prior topical application.

Experimental Protocol for Anticarcinogenesis : One day before the commencement of the experiment, hair on the interscapular region of the mice were shaved. Only the mice showing no hair growth were selected for the study. The animals were randomly allocated into 8 groups comprising of six mice each. The treatment was provided topically on shaved area as described by Berenblum, (1975) and standardized by Agrawal et al (2009) and Sonam and Agrawal (2010)

Treatment Groups

Group 1 (Vehicle control): 100 μl acetone 2 times /week up to 16 weeks

Group 2 (DMBA Alone): - 100 μg DMBA was dissolved in 100 μl acetone and single application was given.

Group 3 (Croton Oil Alone): - 1 % Croton oil was applied on skin 2 times a week up to 16 week.

Group 4 (DMBA + Croton Oil): - 100 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 5 (*Andrographis paniculata* leaves extract Alone): - *Andrographis* leaves extract was topically applied to skin at the dose of 3000 mg/kg b. wt up to 16 week.

Group 6 (DMBA + *Andrographis paniculata* leaves extract + Croton Oil): - Single application of DMBA to skin at the dose of 100 μg DMBA in 100 μl acetone afterwards the 100 μl dose of *Andrographis paniculata* extract at the dose of 3000 mg/kg b. wt. was given one hour before the each application of 1% croton oil twice a week up to 16 weeks.

Group 7 (*Andrographis paniculata* whole plant extract Alone): - *Andrographis* whole plant extract was applied to skin at the dose of 3000 mg/kg b. wt up to 16 week.

Group 8 (DMBA + *Andrographis paniculata* whole plant extract + Croton Oil): - Single application of DMBA at the dose of 100 µg in 100 µl acetone afterwards the 100 µl dose of *Andrographis paniculata* whole plant extract at the dose of 3000 mg/kg b. wt. was given one hour before the each application of 1% croton oil twice a week up to 16 weeks. The animals of all groups were kept under observation for gross and microscopic changes in skin.

The following parameters were observed : Body weight, tumor incidence: cumulative number of papillomas: tumor yield .

And tumor burden:

Biochemical Study: Biochemical alterations were studied in all the groups at the time of termination of the experiment (i.e., at 16th week). The hepatic level of glutathione (GSH) was determined by the method of Moron *et al.* (1979). The GSH content in blood was measured spectrophotometrically using Elman's reagent with 5-5, dithiobis-2-nitrobenzoic acid (DTNB) as a reagent, according to the method of Beutler *et al.*, (1963).

MTT Assay

Extract of whole plant and leaf of *Andrographis paniculata* on Hela cell line was determined by MTT assay as described earlier (Mosmann, 1983). For MTT assay, Hela cells were cultured and treated with different concentrations of extract (0-1000 µg/ml, 5µl/100ul of cell suspension) for 24 hours. 2 hours prior to the termination of experiment, MTT was added to cell culture at 0.25 mg/ml (5µl of 5mg/ml in 100µl of cell suspension) concentration. At the end of the experiment, culture supernatant was removed and cell layer was dissolved in DMSO and further read in a plate reader (BioTek Instruments Inc, Vermont, USA) at 550 nm and 660 nm.

Result

The result of the anticarcinogenic studies have been summarized in Tables 1 and 2. Single topical application of DMBA followed by croton oil, produced skin papillomas, which started appearing from the sixth week onward. The tumor incidence in the DMBA + croton oil treated mice (carcinogen control) reached 100% by the end of the experiment

(16 weeks). The cumulative number of papillomas in carcinogen control mice was recorded as 35. The average number of papillomas per mouse (tumor yield) as well as the papillomas per papilloma-bearing mice (tumor burden) was found to be 5.83 which was reduced in the group which received the treatment of *A. Paniculata* leaves and whole Plant extracts at the dose at 3000 mg/kg body weight (groups VI and VIII). The tumor incidence in *Andrographis* leaves and whole plant extract groups was found to be 66.66% and 83.33% by the end of the experiment (16 weeks). The values of cumulative number of papillomas and tumor yield were recorded as 17 and 21 and 2.66 and 3.5 respectively. Vehicle Control, *Andrographis paniculata* Leaves extract alone and Whole Plant extract alone, Croton oil alone and DMBA alone groups did not induced any tumor incidence.

The increased glutathione (GSH) activity was noticed in blood and liver in the *Andrographis paniculata* Leaves and Whole Plant extract animals as compared to carcinogen control animals (Table 2).

The cytotoxicity of the plant was determined by Hela Cell lines in Vitro model. The highest non cytotoxic dose of whole plant extract of *Andrographis paniculata* after 24 hours of treatment was observed 25µg/ml by MTT assay . Whole plant extract of *Andrographis Paniculata* at highest concentration (500µg/ml) showed 69 % cell death. The highest non cytotoxic dose of leaf extract of *Andrographis paniculata* after 24 hours of treatment was observed to be 250µg/ml by MTT assay (Fig. 2). Leaf extract of *Andrographis Paniculata* showed 74% cell death at concentration of 1000 µg/ml (Fig 1 & 2).

Figure 1: Effect of whole plant extract of *Andrographis Paniculata* on HeLa cell line for 24h by MTT Assay

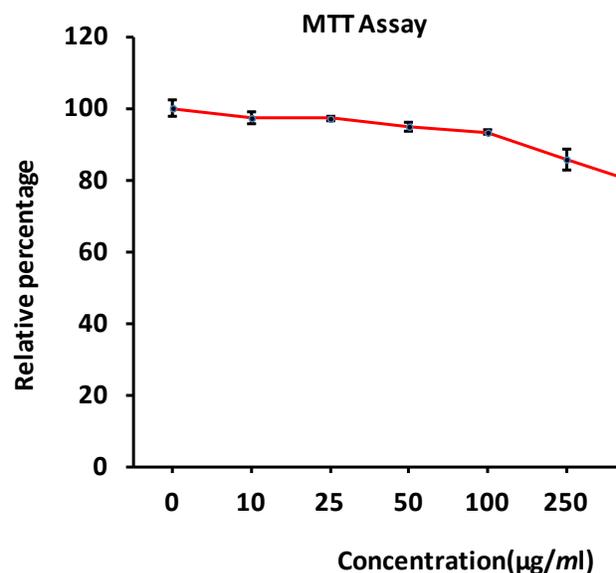
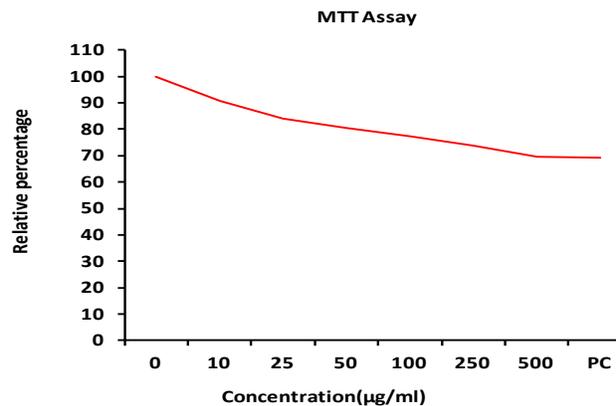


Figure 2: Effect of leaf extract of *Andrographis paniculata* on HeLa cell line for 24h by MTT Assay

Discussion

The present studies demonstrates anticarcinogenic and cytotoxic potential of *Andrographis paniculata* extract for DMBA-induced skin tumorigenesis and in HeLa cells in Vitro Skin carcinogenesis model in experimental animals has been found to be a useful when for investigating the chemopreventors influences both mechanistically and operationally (Morse EC, Stoner G. 1996). Topical application of TPA (active constituent of croton oil) has been reported to increase production of free radicals (Huachen et al., 1991). This is perhaps due to

the free radical oxidative stress that has been implicated in the pathogenesis of a wide variety of clinical disorders (Das, U.N., 2002). Hydro-alcoholic extract of *A. paniculata* was reported by Singh RP *et al.* and indicated the chemopreventive potential of *A. paniculata* against chemotoxicity on drug metabolizing enzymes, antioxidant enzymes, glutathione content, lactate dehydrogenase (LDH), and lipid peroxidation in the liver of Swiss albino mice. A positive anticancer and immunomodulatory activity of the methanolic extract were also reported by Kumar *et al.* (2004) for human cancer cells. 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 (Kausar H, et al., 2003; Sancheti G et al., 2005; Kumar M et al., 2006). There are few reports on the cytotoxic and antiproliferative effects of *Andrographis paniculata* up on in vitro cell lines. It also increased expression of p53, bax and caspase-3 and decreased bcl-2 expression as shown by immunohistochemical analysis was reported (Harjotaruno et al., 2007). All these data suggest that *Andrographis paniculata* as a novel, potential agent in the area of cancer chemoprevention. Further research is required in this direction.

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Table 1.- Effect of *Andrographis paniculata* Leaves and Whole Plant extract on Cumulative No. of Papilloma in Swiss albino mice

S.No.	Groups	Body Weigh (Mean +_SEM)		1 st appearance of Papilloma (In days)	Cumulative No. of Papilloma	Tumor Incidence (%)	Tumor Burden	Tumor Yield
		Initial	Final					
I	Vehical alone	27.78±1.06	31.48±0.94	0	0	0	0	0
II	DMBA alone	27.03±0.97	30.5±1.08	0	0	0	0	0
III	Croton Oil alone	26.95±1.53	30.43±1.41	0	0	0	0	0
IV	DMBA +CO(Control)	27.41±0.76	30.4±0.80	37	35	100	5.83	5.83
V	A.P. Leaves extrat alone	27.31±1.10	30.93±0.71	0	0	0	0	0
VI	DMBA +A.P.Leaves +CO	27.05±1.20	28.18±1.47	58	17	66.66	4.25	2.66
VII	A.P. Whole Plant extrat alone	26.73±1.14	30.08±1.04	0	0	0	0	0

VIII	DMBA +A.P. A.P.Whole Plant extrat +CO	26.08±2.15	27.13±2.23	53	21	83.33	4.20	3.5
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Table2 -The level of Glutathione (GSH) in Blood and Liver of Swiss albino mice(DMBA-induced Papilloma) treated with *Andrographis paniculata* Leaves and Whole Plant extract

S.No.	Groups	Glutathione (GSH)	
		Leve lBlood (µg/ml)	Liver (µg/ml)
I	Carcinogen Control (DMBA + CO)	1.65±0.04	14.95±9.75
II	DMBA +A.P.Leaves extrat +CO	2.12±0.42	46.33±28.36
III	DMBA +A.P. A.P.Whole Plant extrat +CO	3.27±0.05	45.07±44.79