

Chemopreventive effect of *Gymnema sylvestre* in Swiss albino mice

Agrawal, R.C, Soni, S, Jain, N , Rajpoot,J and Maheshwari,S.K

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

Abstract- *Gymnema* is popularly known as gurmar or Madhunashini (destroyer of sugar), as chewing the leaves causes a loss of sweet taste. *Gymnema sylvestre* leaves extract against 7, 12 - dimethylbenz (a) anthracene (DMBA) induced papillomagenesis in Swiss albino mice was studied. The methanolic extract of *Gymnema sylvestre* was 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 average latent period in mice treated topically with *Gymnema sylvestre* extract as compared to the control group treated with DMBA and croton oil. The antioxidant and antibacterial effect of *Gymnema* extract was also observed. The above studies reveal information about the prevention of cancer. Therefore, the present study is immensely important in future drug development programs for the cancer treatment.

Index Terms- papilloma, DMBA, croton oil, In-vivo antioxidant, Antibacterial

I. INTRODUCTION

Cancer is a major killer disease against which no unified treatment concept has emerged so far. Surgery, radiotherapy and chemotherapy often remain the methods of choice in the treatment of cancer. A major hurdle in treating cancer patients using chemotherapy is the severe side effects. Radiation produces reactive free radicals, which cause DNA damage leading to cell death and genomic damage in the stem cells. Antioxidants, which can scavenge the free radicals, are considered for chemo protector. The failure of research efforts to obtain more effective and low cost chemo protector drugs using the synthetic compounds has turned the focus of research towards the natural products in the past decade. Most cancer prevention research is based on the concept of multistage carcinogenesis initiation→ promotion→ progression (Pitot,1991,Morse,1993). In contrast to both the initiation and progression stages, animal studies indicate that the promotion stage occurs over a long time period and may be reversible, at least early on. Therefore, the inhibition of tumor promotion is expected to be an efficient approach to cancer control (Sporn1976 Ind Murkami 1996). 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 radioprotector. 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. *Gymnema Sylvestre* is a woody, climbing plant of tropical forests of central and southern India and in parts of Africa. *Gymnema* has played an important role in Ayurvedic medicine for centuries. *Gymnema* is popularly known as gurmar or Madhunashini (destroyer of sugar), as chewing the leaves causes a loss of sweet taste. (Gloria *et al* 2003). Gymnemic acid, extracted from leaves and roots of *G.sylvestre* is mainly used in India and parts of Asia as a natural treatment for diabetes as it helps to lower and balance blood sugar levels. The major chemical constituents of *Gymnema* are gymnemic acid and gurmarin.

Gymnemic acids have antidiabetic, anti sweetener and anti-inflammatory activities. The leaves of this plant showed the pharmacological activity i.e. Antidiabetic, antimicrobial, antibiotic, anti-inflammatory, *Few studies showed that G. Sylvestre* leaves to cause hypoglycemia in laboratory animals and shown use in herbal medicine to treat diabetes mellitus in adults [Kanetkar *et al*, 2004]. Preliminary phytochemical screening and in vitro anti-oxidant of *Gymnema sylvestre* R.Br. leaf extract were reported (Rachh *et al*, 2009). *Gymnema sylvestre* was also reported to have antidote property against snake venom and was tabulated under the list. Its activity was thought to be due to presence of gymnemgenin. (Walter *et al*, 2000). It has been reported that the gurmarin peptide block the ability to taste sweet or bitter flavors and thus reduces sweet cravings. (Preuss *et al*, 2004). The wound healing activity of carbopol gels prepared from hydro alcoholic extracts of *Gymnema sylvestre* were checked by excision wound model and burn wound models in albino mice. (Kiranmai *et al*, 2011). *In vitro*, the inhibitory effects of DPPH radicals and LDL oxidation were found with aqueous extract of *G. sylvestre*. (Ohmori *et al*, 2005). *In vivo* studies of aqueous extract of GSE containing Gymnemic acids have shown muscle relaxant properties (Luo *et al*, 1999). *Gymnema* preparations have shown to possess anti-allergic activity (Sawabe *et al*, 1992). Aqueous extract of GSE have been shown to be significantly effective in controlling *Culex* larvae. (Tandon *et al*, 2010).

The plant was investigated for immunomodulatory activity by assessing neutrophil locomotion, chemotaxis test, phagocytosis of killed *Candida albicans* and nitroblue tetrazolium tests (Jitender *et al*, 2009). The radio protective effect of Gymnemic acid was evaluated on Swiss albino mice induced by radiation. (Bhatia *et al*, 2008). *Gymnema sylvestre* leaves was investigated of anti inflammatory activity in rats at a dose of 200,300 and 500mg/kg in carageenan induced paw edema and cotton pellet method. (Jitender *et al*, 2008).

One study reported the anticancer activity of *Gymnema sylvestre* on MCF 7 (epithelial cells of human breast cancer) and A 549 (epithelial cells of human lung cancer) under in vitro conditions by MTT assay method (Srikanth *et al*, 2010). Since there is no sufficient report about the anticancer activity of *Gymnema sylvestre* extract, we have therefore evaluated using the two stage skin carcinogenesis model in Swiss albino mice.

II. MATERIALS AND METHODS

2.1 Chemicals

7, 12 - Dimethylbenz (a) anthracene (DMBA), croton oil were purchased 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 104ug/100ul and 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 for the experiments. These animals were housed in polypropylene cages in the animal house at temperatures of $22 \pm 3^\circ\text{C}$. The animals were provided with standard mice feed and tap water ad libitum.

2.3 Preparation of *Gymnema sylvestre* extract Plant material (*Gymnema sylvestre*) was collected locally and identified and the specimen was authenticated at Department of Botany, Safia college, Bhopal (MP), India. . The leaves were washed, air dried, powdered and extracted separately, with 50 % methanol using separating funnel. Extract thus obtained were vacuum evaporated to make it in powder form. These extract was again dissolved in DDW just before topically application.

2.4 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 our previous modified method of Berenblum (1947) reported by us (2009). The animals were randomly allocated into 7 groups comprising six mice each. The treatment was provided topically on shaved area

2.4.1 Treatment Groups

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

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

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

Group IV (*Gymnema sylvestre* Extract Alone): - was applied on skin at the dose of 500 mg/kg b.wt. , 2 times a week up to 16 week.

Group V (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 VI (DMBA + *Gymnema sylvestre* Extract + Croton Oil): -

104 μg DMBA was dissolved in 100 μl acetone and single application was given afterwards the 100 μl dose of *Gymnema sylvestre* extract at the dose of 500 mg/kg b. wt. dose was given one hour before the 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 examined once a week for skin papillomas and these were recorded. The following parameters were taken into consideration:

2.4.2 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.

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

$$\text{Average latent period} = \frac{\sum fx}{n}$$

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.

Anti-bacterial activities

Antibacterial activities of hydro-methanolic extract from leaves of *Gymnema sylvestre* was 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. 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 37°C for 24 hrs.

Concentration

4 different concentrations of crude extract were prepared (100%, 75%, 50%, 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, 25%= 25mg in 1ml.

Study parameter

Measurement of Zone of Inhibition (In mm).

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. The Hydroxyl radical attacked to de-oxyribose and initiated a series of reaction that eventually resulted in the formation of Thiobarbituric Acid Reaction Substances (TBARS). Ascorbic acid= 1mg in 1ml, *Gymnema sylvestre*

Formula:

% Inhibition = $\frac{\text{Abs. 532nm control} - \text{Abs. 532nm test}}{\text{Abs. 532nm control}} \times 100$

III. RESULTS AND DISCUSSION

The phytochemical screening of *Gymnema sylvestre* extract studied showed that the leaves were rich in flavonoids, Phenol, saponins, and tannins. They were known to show medicinal activities as well as exhibiting physiological activities. Tannins and flavanoides have been shown to have numerous health protective benefits, which include lowering of blood lipids.

3.1 Effect of *Gymnema sylvestre* extract on DMBA induced skin Papillomagenesis:

The findings of the present study are depicted in Tables I. Animals of Group- V (control) in which a single topical application of DMBA, followed by croton oil produced skin papillomas, which started appearing 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).

In the skin papilloma model, significant prevention of tumor incidences was observed in the *Gymnema sylvestre* extract treated experimental groups (66% in group VI) as compared to carcinogen control (100%) group. The cumulative number of papillomas was also reduced in the *Gymnema sylvestre* leaves extract treated experimental groups (20 in group VI) as compared to carcinogen control (47) group. The tumor burden and tumor yield were significantly decreased (4.0) as compared to DMBA treated control (7.8) group.

3.2. Anti-bacterial activities:

The Zone of Inhibition (In mm)- *Gymnema sylvestre* extract exhibited strong antibacterial activities for both strain [gram (+) and gram (-) bacteria]. The diameter of zone of inhibition in different standard drugs and different concentration

of *Gymnema sylvestre* is shown in Table 2. No strain in this study showed resistance for this extract and the inhibitory zone is significantly increase in dose depending manner. 50% methanolic extract of leaves of *Gymnema sylvestre* at the different concentration i.e. 25%, 50%, 75%, 100% exhibited antibacterial against and *E. coli*, *Klasebella*, *Staphylococcus* and *Psuedomonas*.

3.3. Antioxidant Activity

The in vitro antioxidant activity of *Gymnema sylvestre* leaves was tested in various concentrations against Ascorbic acid as standard. Percentage of TBARS was calculated for both Ascorbic acid and *Gymnema sylvestre* extract, with the help of formula, for a comparative study. In vitro antioxidant activities of *Gymnema sylvestre* extract showed significant inhibitory concentration as compared to ascorbic acid.

Discussion:

Gymnema has played an important role in Ayurvedic medicine for centuries. Gymnemic acid, extracted from leaves and roots of *G. sylvestre* is mainly used in India and parts of Asia as a natural treatment for diabetes as it helps to lower and balance blood sugar levels. The aqueous extract of *Gymnema sylvestre* leaves was reported anti-inflammatory in rats (Jitender *et al*, 2008) and in one study reported the anticancer activity of *Gymnema sylvestre* on MCF 7 (epithelial cells of human breast cancer) and A 549 (epithelial cells of human lung cancer) under in vitro conditions by MTT assay method (Srikanth *et al*, 2010). In our study, the *Gymnema sylvestre* leaves extract showed significant antitumor activity in skin Papilloma model. It also showed antibacterial and antioxidant activities in Vitro test systems. The above studies reveal information about the prevention of cancer. Therefore, the present study is immensely important in future drug development programs for the cancer treatment.

IV. CONCLUSION

The *Gymnema sylvestre* extract have shown significant antitumor, antibacterial and antioxidant activities. It may be an important drug for prevention of bacterial infection, prevention of cancer and scavenging of hydroxyl radicals which are generated during carcinogenesis.

REFERENCES

- [1] Agrawal, R.C., Jain, R., Wasim Raja, Ovais, M. (2009): Anticarcinogenic Effects of Solanum lycopersicum Fruit Extract on Swiss Albino and C57 BL Mice. Asian Pacific Journal of Cancer Prevention.10:379-381.
- [2] Berenblum, J., Shubik, P. (1947): A new quantitative approach to the study of stages of chemical carcinogenesis in the mouse skin. Br. J. Cancer. 1:373-391.
- [3] Bhatia AL, Raka K, Gulshan V, Sharma KV, Sharad V, Megha J (2008). Radio protective role of Gymnemic acid on mice: Study on Hepatic Biochemical Alterations. Asian J. Exp.Sci; 22(3):427-32.
- [4] Gloria Y Yeh, David M. Eisenberg, Ted J. Kaptchuk, Russell S. Phillips (2003). Systematic Review of Herbs and Dietary Supplements for Glycemic Control in Diabetes. Diabetes Care; 26 (4): 1277-1294.
- [5] Jitender KM, Manvi FV, Alagawadi KR and Noolvi M (2008): Evaluation of anti-inflammatory activity of *Gymnema sylvestre* leaves extract in rats. Int. J. Green Pharmacy: 114-115.

- [6] Jitender KM, Manvi FV, Nanjwade BK, Alagawadi KR and Sanjiv S (2009): Immuno - modulatory activity of *Gymnema sylvestre* leaves extract on in vitro human neutrophils. *J. Pharmacy Res*; 2(8):1284-6.
- [7] Kanetkar, P.V. Laddha, K.S. Kamat. M.Y (2004). *Gymnemic acids: A molecular perspective of its action on carbohydrate metabolism*. Poster presented at the 16th ICFOST meet organized by CFTRI and DFRL, Mysore, India.
- [8] Kiranmai M, Kazim SM and Ibrahim M (2011). Combined wound healing activity of *Gymnema sylvestre* and *Tagetes erecta* Linn. *Int. J. Pharmaceu. Appl.*; 2(2):135-140.
- [9] Luo H (1999). Possible participation of NO and EDHF in the relaxation of rat intestinal circular muscle induced by *Gymnema* water extracts containing gymnemic acids. *Journal of the Yonago Medical Association*; 50(1): 22-31.
- [10] Morse MA, Stoner GD. *Cancer chemoprevention: principles and prospects*, *Carcinogenesis*. 1993; 14(9) :1737-1746
- [11] Murakami A, Ohigashi H, Koshimizu K. *Anti-tumor promotion with food phytochemicals: a strategy for cancer chemoprevention*, *Bioscience, Biotechnology, and Biochemistry*. 1996; 60(1) :1-8,
- [12] Ohmori, R Iwamoto, T Tago, M Takeo, T (2005). Unno, T Itakura H. *Lipids*, 40 (8), 849-53.
- [13] Pitot HC, Dragan YP, *Facts and theories concerning the mechanisms of carcinogenesis*, *The FASEB Journal*. 1991; 5(9) :2280-2286
- [14] Preuss, H.G. Bagchi, (2004). D Bagchi, M. Rao, C.V. Dey, D.K Satyanarayana. *S. Diabetes Obes Metab*, 6(3), 171-80.
- [15] Rachh PR, Patel SR, Hirpara HV, Rupareliya MT, Rachh MR, Bhargava AS, Patel NM and Modi DC (2009): In-vitro evaluation of antioxidant activity of *Gymnema sylvestre* R.Br. leaf extract. *Rom. J.Biol.Plant Biol.*; 54(2):141-8.
- [16] Sawabe Y, Nakagomi K, Iwagami S, Suzuki S, Nakazawa H (2009). Inhibitory effects of pectic substances on activated hyaluronidase and histamine release from mast cells. *Biochim Biophys Acta* 1992; 1137(3): 274-278.
- [17] Sporn MB, *Approaches to prevention of epithelial cancer during the preneoplastic period*, *Cancer Research*. 1976;36(7): 2699-2702,
- [18] Srikanth AV, Sayeeda M, Lakshmi NM, Ravikumar P and Madhava RB (2010): Anti cancer activity of *Gymnema sylvestre* R.Br. *Int. J. Pharmaceu. Sci. Nanotech*; 3(1):897-899.
- [19] Tandon Pankaj, Sirohi Anita (2010). Assessment of larvicidal properties of aqueous extracts of four plants against *Culex quinquefasciatus* larvae. *JJBS*; 3(1):1-6.
- [20] Walter BM, Maria CDN, Bettina MRP and Nuno AP (2000). Plant natural products active against snake-bite-the molecular approach. *Phytochemistry*; 55:627- 642.

AUTHORS

First Author – Dr.R.C.Agrawal ,M.Sc. & Ph.D. Professor & Head ,Research department, Priyamvada Birla Cancer Research Institute,Satna, M.P..

Second Author – Ms. Sonam Soni, , M.Sc.Research scholar, Research department, Priyamvada Birla Cancer Research Institute,Satna, M.P..

Third Author – Ms Nishtha Jain,B.Tech Research Officer Research department, Priyamvada Birla Cancer Research Institute,Satna, M.P..

Fourth Author, Mr. Jitendra Rajpoot, M.Sc, Animal supervisor cum Lab Technician Research department, Priyamvada Birla Cancer Research Institute,Satna, M.P..

Fifth Author Dr.S.K.Maheshwari: M.B.B.S.& M.S., Medical Director,PBCRI,Satna,M.P.

Correspondence Author – Dr. R. C. Agrawal, rcagrwal60@yahoo.com,919826949427

Table No1: Showing Cumulative No. of Papilloma in different group.

S.No.	Groups	Dose	Time of 1 st appearance of Papilloma	Cumulative No. of Papilloma	Mean No. of Papilloma	Tumour Yield
I	Vehicle alone	100µl/animal	–	–	–	–
II	DMBA alone	104µg/animal	–	–	–	–
III	Croton Oil alone	1% per animal	–	–	–	–
IV	<i>Gymnema sylvestre</i> Extract alone	mg/kg per animal	–	–	–	–
V	DMBA + CO (Control)	58 th Day	47	47/6	6/6 (100%)	7.8

VI	DMBA+CO+ <i>Gymnema sylvestre</i> Ext.	82 th Day	20	20/5	4/6 (66 %)	4.0
----	--	----------------------	----	------	----------------	-----

TABLES 2 Antibacterial activity of *Gymnema sylvestre* against bacterial strains

Name of microorganisms	% Concentration of Extract [zone of inhibition(mm)]			
	25	50	75	100
<i>Klebsiella</i>	10	12	13	15
<i>E. Coli</i>	13	14	13	15
<i>Staphylococcus</i>	16	17	16	15
<i>Psuedomonas</i>	-	-	11	12

Table no.3 In vitro antioxidant activity of 50% methanolic *Gymnema sylvestre* extracts Vs Ascorbic acid (standard)

Sr. No.	Concentration of ascorbic acid (µg)	% TBARS inhibition SEM	Concentration of <i>G. Sylvestre</i> (µg)	% TBARS inhibition SEM
1	30	25.29	10	22.608
2	60	207.44	20	13.913
3	90	266.07	30	100
4	120	438.09	40	176
5	150	393.75	50	31.304
6	180	411.01	60	198.26
7	210	645.83	70	159.56
8	240	651.19	80	97.82

9	270	388.09	90	246.52
10	300	745.23	100	206.95