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 tropically 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. Sylvester 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 alcholic 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 104μg/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 ± 3°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 sylvester 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

Average latent period = Σfx/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.

Antibacterial 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 Thiobarbeturic Acid Reaction Substances (TBARS). Ascorbic acid = 1mg in 1ml, Gymnema sylvestre extract

Formula:
% Inhibition = Abs. 532nm control - Abs. 532nm test Abs./532nm control x 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 flavonoids 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, Klabsella, 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 Gymnemista 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 antitumour 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 antitumour, 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
Table No1: Showing Cumulative No. of Papilloma in different group.

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

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.

Forth 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, rcaagrawal60@yahoo.com, 919826949427
### TABLES 2  Antibacterial activity of *Gymnema sylvestre* against bacterial strains

<table>
<thead>
<tr>
<th>Name of microorganisms</th>
<th>% Concentration of Extract [zone of inhibition(mm)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>10</td>
</tr>
<tr>
<td><em>E. Coli</em></td>
<td>13</td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>16</td>
</tr>
<tr>
<td><em>Psuedomonas</em></td>
<td>-</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Concentration of ascorbic acid (µg)</th>
<th>% TBARS inhibition</th>
<th>SEM</th>
<th>Concentration of <em>G. Sylvestre</em> (µg)</th>
<th>% TBARS inhibition</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>25.29</td>
<td></td>
<td>10</td>
<td>22.608</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>207.44</td>
<td></td>
<td>20</td>
<td>13.913</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>266.07</td>
<td></td>
<td>30</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>120</td>
<td>438.09</td>
<td></td>
<td>40</td>
<td>176</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>393.75</td>
<td></td>
<td>50</td>
<td>31.304</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>180</td>
<td>411.01</td>
<td></td>
<td>60</td>
<td>198.26</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>210</td>
<td>645.83</td>
<td></td>
<td>70</td>
<td>159.56</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>240</td>
<td>651.19</td>
<td></td>
<td>80</td>
<td>97.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>270</td>
<td>388.09</td>
<td>90</td>
<td>246.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>300</td>
<td>745.23</td>
<td>100</td>
<td>206.95</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>