Chemopreventive and anticarcinogenic effect of Kamdhenu Ark in experimental mice

Agrawal, R.C1 Padole, S2 and Maheshwari, S.K3

1Department of Research, Priyamvada Birla Cancer Research Institute, Satna
Madhya Pradesh, India
2Jawaharlal Nehru Cancer Hospital, Bhopal

Abstract:
The aim of study to evaluate anticarcinogenic and antimutagenic activity of Kamdhenu Ark (DCU) in experimental animal. The anticarcinogenic activity was evaluated using 2 stage protocol of mouse skin carcinogenesis and melanoma tumour model. The antimutagenicity was studied by the prevention of micronucleus formation in bone marrow cells of mice.

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). Significant prevention of tumor incidences was observed in the Kamdhenu Ark (DCU) treated experimental groups (48% & 30% in group V & VI) as compared to carcinogen control (100%) group. The cumulative number of papillomas was also reduced in the Kamdhenu Ark treated experimental groups (14 & 19 in group V & VI) as compared to carcinogen control (27) group. In melanoma tumour model, In group I no treatment was given and observed 100% tumour incidence. In group II cyclophosphamide was given at the dose of 80 mg/kg. The tumour size was observed 1774 mm2 as compared to group III and IV 1509 and 910 mm2 which received the treatment of DCU alone and DCU + cyclophosphamide at the dose of 0.2 ml/mice. The induction of micronuclei formation by cyclophosphamide was significantly inhibited when the animals were given single application of DCU (distilled cow urine) at the different volume of 0.2, 0.4 & 0.6 /mice in different group of mice 24 hours before the cyclophosphamide treatment. A single application of DCU at the dose of 0, 8 ml/mice has no effect of the induction of micronuclei as compared to solvent control. In our study the tumour preventive effect of Kamdhenu Ark was observed in Papilloma & melanoma tumour model. The prevention of micronucleus formation was also observed in bone marrow cells of mice. The present study may advocate the use of Kamdhenu Ark as adjuvant in the chemoprevention of Cancer.

Keywords: papilloma, DMBA, Melanoma, Micronucleus.

INTRODUCTION:
“Panchagavya” is a combination of cow urine, milk, dung, ghee and curd. The ancient Indian system of medicine, Ayurveda, has detail mentions of importance of panchagavya in the treatment of various human diseases. Apart from high medicinal values, panchagavya has many beneficial implications in agriculture, organic farming as good quality natural manure and biopesticides, bio-fertilizer, pest repellants and as alternate energy resources (biogas, fuel and electricity) (Charaka-Samhita, 1981, Susruta Samhita, 1985; Tietze, 1996; Chauhan, 2002; Fulzele et al., 2001, 2003; Joshi, 2002; Nautiyal, 2002; Garg and Chauhan, 2003a,b; Achliya et al., 2004; Saxena et al., 2004, Singh and Chauhan, 2004 ). The incidences of human sufferings from cancer, a deadly malady, are ever increasing in the current scenario of changed lifestyle and food habits along with dangers of carcinogen in the form of tobacco chewing, smoking, alcohol intake, environmental pollutants, occupational health hazards etc. The methodologies like chemotherapy, radiotherapy and surgery are available for treating the cancer but the success rate is low particularly with malignant tumors, and moreover, these therapies produce severe side
effects adding to the physical and mental agony of the patient and are costly, and even some of the tumors are incurable. In all these circumstances mentioned above one has to think over the alternative therapeutic approaches to control the infections, save mankind from different ailments and fight diseases such as cancer (Garg and Chauhan, 2003; Chauhan, 2005). Utilizing the beneficial properties of cow’s urine, milk, ghee, curd and dung, the kind of treatment is called panchgavya therapy or cowpathy, a system of naturopathy. Panchgavya products have been found to be beneficial in curing several human ailments and enhance the body’s immunity and resistance to fight the infections. This kind of alternative treatment has been reported to be beneficial even for dreaded diseases like cancer, AIDS and diabetes. Of these, the cow urine recently has received worldwide attention. Two US patents have been granted to Indian scientists on establishing the bio enhancing properties of cow urine, its use in tuberculosis patients and fight cancers, thus opening a new era in medical science. Cow urine along with the antibiotics can also prevent the development of resistance in microorganisms against the antibiotics.

Cow urine has been described in Sushrta Samhita and, Ashtanga Sangraha to be the most effective substance/secretion of animal origin with innumerable therapeutic values. In India, drinking of cow urine has been practiced for thousands of years. It is an important ingredient of panchgavya, and its immunomodulatory properties have been reported that it enhances both cellular and humoral immune response (Kumar, 2001; Chauhan et al., 2004).

Cow urine constituents are capable of removing the ill effects, imbalances in the body (Bartnett, 1988; Chauhan et al., 2001; Chauhan, 2003a). Cow urine contains 24 types of salts. Its main contents are water 95%, urea 2.5%, minerals, salt, hormones, and enzymes-2.5%. It contains iron, calcium, phosphorus, carbonic acid, potash, nitrogen, ammonia, manganese, iron, sulphur, phosphates, potassium, urea, uric acid, amino acids, enzymes, cytokine and lactose etc. (Bhadauria, 2002). Practitioners of Ayurvedic medicine (from India) routinely use urine as a remedy. A number of ailments could be treated by cow urine therapy. Most of the medicines are made by distilling urine and collecting vapours termed as ‘ark’ (distillate). In poultry, cow urine has been reported to enhance the immune competence of birds and provide better protection along with vaccination and increases egg production and egg quality. In-vivo cow urine treatment to developing chicks marginally up regulated the lymphocyte proliferation activity (Prabhakar et al., 2004). Chauhan et al. (2001) and Kumar et al. (2004) reported the cow urine (Kamdhenu ark” / cow urine distillate) to be a potent and safe immunomodulator, which increases both humoral and cell mediated immunity in mice. It was observed that cow urine enhances both T and B cell proliferation and also increases the titer level of IgG, IgA and IgM antibodies. Chauhan et al. (2004) observed that it increases the secretion of interleukin-1 and 2 also. Prabhakar (2004) reported that the cow urine had protective effect on lymphocytes of birds undergoing apoptosis and suggested the exploitation through experimental trails for specific use of cow urine as an adjunct to vaccination. Thus the cow urine not only maintains the immunity of body but also modulate it in the positive direction to an optimum level. Garg et al. (2004) reported beneficial effects of cow urine on serum biochemical profile (total serum protein, glucose, calcium and cholesterol of laying birds. Its usefulness as antimicrobial agent, positive effect on body weight gain and haematological profiles have also been reported. Garg et al. (2004) suggested that cow urine can be used as a feed additive for layer birds in order to get good quality eggs and immune-enhancer. A cow urine distillate fraction (ark) has been identified as a bioenhancer of the activities of commonly used antibiotics, anti-fungal and anti-cancer drugs. Recently the cow urine has been granted U.S. Patents (No. 6896907 and 6410059) for its medicinal properties, particularly for its use along with antibiotics for the control of bacterial infection and fight against cancers. The activity of Rifampicin, a front-line anti-tubercular drug used against tuberculosis, increases by about 5-7 folds against E. coli and 3-11 folds against Gram-positive bacteria (The Hindu, 4 July, 2002; The Indian Express, 4 July, 2002). Bioenhancers are substances, which do not possess drug activity of their own but promote and augment the bioactivity or bioavailability or the uptake of drugs in combination therapy. Such bioenhancers have been earlier isolated only from plant sources. The people frustrated from the heavy medication of allopathy are now using cowpathy drugs and being benefited by the panchgavya products for several diseases. Cow urine is an important element of panchgavya, and the cow urine therapy has been successful in relieving human sufferings. However, scientific validation of cow urine is required for its worldwide acceptance and popularity in terms of medicinal applications so as to exploit its optimal power for the service of mankind. Regardless of scientific validation, people are using and getting benefits of it.

Materials and Methods:

Chemicals

7, 12 - Dimethylbenz (a) anthracene (DMBA), croton oil, cyclophosphamide were obtained 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.

**Animals**

Random bred male Swiss albino and C57 Bl mice (6 - 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°C. The animals were provided with standard mice feed and tap water ad libitum. The distilled Kamdhenu Ark was obtained from Cow Research Centre, Kerava dam, Bhopal.

**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( ) reported by us (Agrawal et al, 2009 ).The animals were randomly allocated into 6 groups. The study was approved by the Institutional animal ethical committee of PBCRI, Satna,M.P.

**Treatment Groups**

**Group 1 (Untreated control):** No treatment

**Group 2 (DMBA Alone):** - 104 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 (Kamdhenu Ark Alone):** - 100 ul Kamdhenu Ark was applied on skin 2 times a week up to 16 week.

**Group 5 (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 6 (DMBA + Kamdhenu Ark + Croton Oil):** - 104 μg DMBA was dissolved in 100 μl acetone and single application was given afterwards the 100 μl dose of Kamdhenu Ark was given topically 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:

**Tumor study:**

**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 = \( \frac{2fx}{n} \)**

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.

**Melanoma tumor model**

Experiment was performed by the using method of Oommen, et. al. (1999) and Agrawal et al. (2009). Hair of dorsal side in a particular area (1 X 1 cm²) was removed by hair remover cream of each mouse. All mice were kept in a laminar air flow cabinet under pathogen free conditions throughout the tumor implantation. The implanted tumor from tumor donors (mouse having melanoma tumor) was removed from the mice. Tumor was dissected out and removed necrotic tissue and blood vessels was cut into small pieces in PBS, and it was munched with the help of bent scissor. Cell suspension was filtered and 100 μl stained with trypan blue stain. All lives cells which were not stained were counted using haemocytometer. C57BL/6 mice had their dorsal side shaved and were inoculated Subcutaneously (S.C.) with 5 × 10^7 B16(F10) melanoma cells in 100 μl of PBS per animal. The implanted tumors bearing mice were randomly divided into 4 groups with 4 mice in each group.

**Treatment Groups:**

**Group 1 (Untreated Group)**

This group consisted of four mice bearing the melanoma cell line (B16F10). No treatment was given.

**Group 2 (Kamdhenu Ark + Cyclophosphamide Group)**

This group consisted of four animals bearing the melanoma cell line (B16F10). The mice were given Kamdhenu Ark orally at the dose of 500 mg/kg body weight and after 30 min. Cyclophosphamide at the dose
of 170 mg/kg was injected intraperitoneally. During the treatment the size of the implanted tumors was measured by Vernier calipers.

**Group 3 (Kamdhenu Ark alone Group)**

This group consisted of four animals bearing the melanoma cell (B16F10). The animal was given **Kamdhenu Ark orally** and i.p.at the dose of 100 ul/mice during the treatment and the size of the implanted tumors was measured by Vernier calipers. During the treatment, the size of the implanted tumors was measured using Vernier calipers. Tumor volume was calculated.

**Micronucleus Assay:**

Both sex of **Swiss albino** mice of 15-20gm body wt. were obtained from the animal colony of our Research Centre and 4-5 animals were housed in each plastic cage. The animals were provided Standard pellet diet and water **ad libitum**. This assay was performed as per the method reported by Schmid (1975) and modified by Agrawal et al (1998). Test substances applied intraperitoneally and animals were sacrificed by cervical dislocation and bone marrow cells were harvested from freshly killed animal muscles was removed from bone by use of gauze and fingers. Bone marrow cell was aspirated by flushing with HBSS solution with help of a syringe. The tube was centrifuged at 1000 rpm for 5 min. The supernatant was removed the cells in the sediment were carefully mixed by aspiration and a small drop of the viscous suspension was put on the end of a slide and spread by pulling the material behind a polished cover glass held at an angle of 45 degree. The preparation was then dried and fixed for 2-5 min. Staining was carried out in ordinary vertical staining jars according to the following procedure. Stained for 5 min in may-Grunwald solution and stained for 10 min in Giemsa then slides rinsed in distilled water, blotted cleaned back side of slides with filter paper then dried on the slide warmer. The frequency of micronucleated PCE cells was counted in 2000 PCE & NCE cells and compared among treatment groups. Cells which stain uniformly positive for RNA are referred to as polychromatic or polychromatophilic erythrocytes (PCEs). Cells which do not stain positively for RNA are referred to as normochromatrophic erythrocytes (NCEs). An increase in frequency of micro nucleated PCEs relative to the vehicle control group indicates the test substance induced chromosomal damage or lagging chromosome in the nucleated erythrocytes cells.

**RESULTS:**

**Effect of Kamdhenu Ark (DCU) on DMBA induced skin Papillomagenesis:**

The findings of the present study are depicted in Tables I and Fig. 1. 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). Significant prevention of tumor incidences was observed in the Kamdhenu Ark treated experimental groups (48 % and 30 % in group VI and VII) as compared to carcinogen control (100 %) group. The cumulative number of papillomas was also reduced in the Kamdhenu Ark treated experimental groups (14 & 19 in group VI & VII) as compared to carcinogen control (27) group (Table 1)

<table>
<thead>
<tr>
<th>Table 1: Anticarcinogenic activity of Distilled Cow Urine induced by DMBA + Croton Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>III</td>
</tr>
<tr>
<td>IV</td>
</tr>
<tr>
<td>V</td>
</tr>
<tr>
<td>VI</td>
</tr>
<tr>
<td>VII</td>
</tr>
<tr>
<td>VIII</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table No.2 Anticarcinogenic activity of Cow urine in Melanoma tumour model</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.No Groups</td>
</tr>
<tr>
<td>--------------</td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>III</td>
</tr>
<tr>
<td>IV</td>
</tr>
</tbody>
</table>

Effect of DCU in melanoma tumour model: In group I no reatment was given and observed 100 % tumour incidence. In group II Cyclophosphamide (80 mg/kg i.p.) was given. The tumour size was observed 1774 mm 2 as compared to group III and IV 1509 and 910 mm 2 which received the treatment of DCU alone and DCU + cyclophosphamide at the dose of 0.2 ml/mice. The tumour inhibition rate was 48 in group IV as compared to 38 and 15 % in group II & III respectively (Table 2).

Effect of DCU in Micronucleus formation: The induction of micronuclei formation by cyclophosphamide was significantly inhibited when the animals were given single application of DCU (distilled cow urine) at the different volume of 0.2,0.4 & 0.6 /mice in different group of mice 24 hours before the cyclophosphamide treatment. A single application of DCU at the dose of 0.8 ml /mice has no effect of the induction of micronuclei as compared to solvent control ( Table 3 ).

Discussion: In our study the tumour preventive effect of Kamdhenu Ark was observed in Papilloma & melanoma tumour model. The reduction of papillomas was also reported in our previous communication (Raja & Agrawal 2010). The prevention of chromosomal damage and micronucleus formation was also observed in bone marrow cells of mice. When cow urine was given along with cyclophosphamide a Known anticancer drug, the melanoma tumour prevention was more as compared to Cyclophosphamide group. It seems that cow urine acts as a bioenhancer in our tumour model. Cow urine was reported to increases the efficacy of the antibiotics against infectious agents. Bioenhancement has been reported with antibiotics viz. Ampicillin, Isoniazid, Clotrimazole, Cyanocobalamine etc. This activity has been reported to reduce the antibiotic dose per day and duration of treatment in tuberculosis patients (Joshi, 2002). The Indigenous cow urine contains “Rasayan" tatva, which may be responsible to modulate immune system and act as bioenhancer. A U.S. Patent (No. 6896907) was granted to Indian scientist for use of cow urine in cancer patients.(Amar Ujala, July, 19, 2005). Distilled cow urine protects DNA damage and repairs it rapidly as reported after damage due to pesticides (Ambwani, 2004). It protects chromosomal aberrations by mitocycin in human leukocyte (Datta, 2001). Cow urine helps the lymphocytes to survive and not to commit suicide (apoptosis). Kumar et al. (2004) reported the prevention of pathogenic effect of free radicals through cow urine therapy. These radicals are reported to damage to various tissues and attack enzymes, fat and proteins disrupting normal cell activities or cell membranes, producing a chain reaction of destruction leading to the ageing process of a person. Its protection may be possible by regular use of cow urine. The cow urine may be advocated to use adjuvant to chemotherapy for cancer treatment. Further clinical trial are required.

REFERENCES
Agrawal, R.C and Kumar, S. Prevention of cyclophosphamide induced Micronucleus formation in mouse bone marrow by Indole-3-carbinol, Food and Chemical Toxicol, 1998, 36, 975-977.
Chauhan, R.S. (2003c). Neoplasms, in Illustrated Special Veterinary Pathology, RS Chauhan (Editor), IBDCO Publishing Division, Lucknow, India. Chapter 1, 1-67.


Raja , W and Agrawal, R.C. Chemopreventive potential of Cow urine against 7, 12-dimethylbenz(a) anthracene induced skin papilligenesis in mice. Academic journal of Cancer Research Vol. 3 (1), 7-10, 2010


Susruta Sanhita - The Medical Science of the Ancient Aryans”, Tr. and Ed. A.C. Bandopadhyaya, 2nd ed. Calcutta. 1885. 10


The Hindu, 4 July, 2002. United States Patent and Trade Office had granted Patent No 6410059 to an "Indian innovation which has proved that cow's urine can make antibiotics, anti-fungal agents and also anti-cancer drugs more effective”.

The Indian Express 4 July 2002, Central Institute of Medicinal and Aromatic Plants at Lucknow established that "Certain compounds in cow urine, when used in combination with certain antibiotics like the commonly used anti-tuberculosis drug rifampicin, can help kill more bacteria than a single application of the antibiotic".

---

**Cite this article as:**
