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Chemopreventive Potential of Cow Urine Against 7,12-dimethylbenz(a) anthracene-induced Skin Papillomagenesis in Mice

Wasim Raja and R.C. Agrawal

Department of Research, Jawaharlal Nehru Cancer Hospital and Research Centre Idgah Hills, Bhopal, 462001 Madhya Pradesh India

Abstract: Cow, *Bos indicus* is a most valuable animal in all Veda and it is called as the Mother of all. From the ancient period cow's urine has been used as a medicine and it was compared to the nectar in Veda. In Susrut, several medicinal properties of cow's urine have been mentioned and in Indian tradition cow urine or Go- mutra is believed to have therapeutic properties. In the present investigations, the anticarcinogenic activity of cow's urine was evaluated using two stage in *Swiss albino* mice, induced by a single application of 7, 12-dimethyabenz(a)anthrecene (104 μg/ 100 μl acetone) and one weeks later, promoted by repeated application of croton oil (1% in acetone/thrice a week) till the end of the experiment (16 weeks). The tumor incidence, tumor yield, tumor burdon and cumulative number of papillomas were found to be higher in the control (without cow urine treatment) as compared to experimental animals (cow urine treated). The differences in the values of the results of experimental groups were statistically analysed and found to be significant in comparison to the control group ($p< 0.05$). In conclusion, the present study demonstrates the chemopreventive potential of cow urine on DMBA induced skin tumorigenesis in *Swiss albino* mice.

Key words: Chemopreventive · Cow urine · Papilloma · Skin carcinogenesis

INTRODUCTION

Traditional medicines, whether from Ayurveda or Siddha are based on classical texts and systems, practices and products handed down over generations going back to Charak, Sushrutha, Vagabhatta, the Ashtangahridaya and the Samhitas. Several medicinal properties of cow's urine have been mentioned and in Indian tradition cow urine is believed to have therapeutic properties [1]. Cow urine acts as bioenhancer and increased the efficacy of the antibiotics against the infectious agent with drugs viz. ampicillin, isonized, clotrimazole etc. [2]. The cow urine are reported to contain 24 types of salt and the medicine made from the cow urine which is used to cure various diseases [3, 4]. The antigenotoxic/antioxidant properties of cow's urine distillate and redistillate were reported. *In vitro* [5] Kamdhenu Ark (Cow Urine distillate) decreases the toxic effect of cadmium chloride in mice [6]. The immunomodulatory effect of Kamdhenu Ark was also observed in mice [7]. It can cure disorders without inducing toxicity and have been reported to enhance cellular and humeral immune response [8]. Prabhakar, [9] reported that cow urine helps the lymphocytes to survive and to repair the damaged DNA and thus it is effective for cancer therapy [10]. The cow urine is protective effect on lymphocytes of birds undergoing apoptosis and suggested exploitation through experimental trails for specific use of cow urine as an adjunct to vaccination. Kumar *et al.* [8] reported the prevention of pathogenic effects of free radicals through cow urine therapy. Gupta *et al.* [11] and Chauhan *et al.* [7] reported beneficial effect of cow urine on serum biochemical profiles (total serum protein, glucose, calcium and cholesterol) of laying birds. Gupta *et al.* [11] also reported the usefulness as antimicrobial agent positive effect on body weight gain, hematological profiles, immunomodulatory effect and healing of surgical wounds in experimental rats. According to ancient literatur distillate of cow urine was the one to be used mainly and the distillate was found to exhibit antioxidant effect [5]. However, there are no reports on the anticarcinogenic property of cow's urine in experimental animals are

Corresponding Author: Wasim Raja, Department of Research, Jawaharlal Nehru Cancer Hospital and Research Centre Idgah Hills, Bhopal, 462001 Madhya Pradesh, India.

available. The present investigation was carried out to study the protective effect of cow urine using two stage skin carcinogenesis protocol in *Swiss Albino* mice.

MATERIALS AND METHODS

Cow Urine: Distilled Cow Urine was obtained from the Kamdhenu Gaushala, Bhopal, Madhya Pradesh, India.

Chemicals: 7, 12, Dimethyl benzanthracene (DMBA) and croton oil was purchased from Sigma chemical Co., U.S.A. DMBA was dissolved at a concentration of 104 μg/100 μl in acetone. Croton oil was mixed in acetone to give a solution of 1% dilution and other chemical were reagents grade and were procured locally for the study.

Animals: The study was conducted on random bred, 6-7 weeks old and 25 ± 2 g body weight bearing, male Swiss albino mice. These were maintained under controlled conditions of temperature (25+2°C) and light (14 light: 10 dark). The animals were fed on standard mice feed procured from Brook Bond Lipton India Limited, Calcutta and water *ad libitum*. Four animals were housed in a polypropylene plastic cage containing saw dust (procured locally) as bedding material. As a precaution against infections, tetracycline hydrochloride water was given to these animals once in a fortnight. The study protocol is approved by the Departmental Animal Ethical Committee and confirms to the guidelines set by World Health Organization, Geneva, Switzerland and Indian National Science Academy (INSA), New Delhi (India).

Experimental Protocol: The animals were randomly divided in to 7 groups. Each group comprises of 10 animals and hair were shaved in 2cm ^{2} area with the help of hair removing cream in interscapular region initially and after every 2 weeks hair were removed with the help of scissors. The treatment was provided topically on shaved area using the following protocol of Berenblum [12].

Treatment Groups:

Group 1 (Untreated control), received no treatment.

Group 2 (Vehicle control): 100 μl acetone 3 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 weeks.

Group 5 (DMBA + Croton Oil): 104 μg DMBA was dissolved in 100 μl acetone and single application was given up afterwards 1 % Croton oil was applied on skin 2 times a week up to 16 weeks.

Group 6 (DMBA + Cow urine + Croton Oil): 104 μg DMBA was dissolved in 100 μl acetone and single application was given afterwards the 100 μl dose of cow urine was given 1 hrs before the each application of 1 % Croton oil 2 times a week up to 16 weeks.

Group 7 (Cow Urine alone): the 100 μl dose of. Cow urine was given 2 times a week up to 16 weeks.

The animals were kept under observation for gross and microscopic changes in skin.

Statistical Analysis: The differences in the incidence of tumors among different groups were evaluated by Student 't' test and considered significant at 5% significance level ($p<0.05$).

RESULTS

The findings of the present study have been depicted in Table 1. The administration of Cow urine did not affect the body weight of the animals during the experimental period. Papillomas started appearing on the skin area from 7-13 weeks during exposure to the initiator and the promoter. Percent inhibition of tumor multiplicity reduced significantly in the experimental groups as compared to control. In carcinogen control group, treated with a single dose of DMBA and one week later promoted by repeated application of croton oil, all animals developed skin papillomas (100% tumor incidence). The average numbers of papillomas per mouse (tumor yield) as well as the papillomas per papilloma bearing mice (tumor burdon) were found to be 2.8. Cumulative numbers of papillomas in these mice were recorded as 17. In the animals of group which receiving similar treatment of DMBA and croton oil when subjected to topical administration of Cow urine, tumor incidence, tumor yield, tumor burdon and cumulative number of papillomas were recorded as 33%, 1.0, 2.0 and 6, respectively.

The difference in the values of the results of Groups were statistically analysed (Table 1) and found to be significant in comparison to the control group ($p< 0.05$).

Table 1: Effect of Cow urine on DMBA-induced skin tumour in *Swiss albino* mice.

S. No.	Group	Body Weight		Tumours incidence	Cumulative number	Tumor yield	Tumor burdon
		Initial	Final				
1.	Untreated Control	25.3 ± 1.3	30.2 ± 1.2	0/6	00	00	00
2.	Vehicle control	26.8 ± 2.0	30.8 ± 2.5	0/6	00	00	00
3.	DMBA alone	24.7 ± 1.1	29.4 ± 1.8	0/6	00	00	00
4.	Croton oil alone	25.6 ± 2.1	29.9 ± 1.0	0/6	00	00	00
5	DMBA+ Croton oil	25.8 ± 1.3	32.0 ± 1.6	6/6 (100%)	17	2.8	2.8
6.	DMBA+ Cow urine + Croton oil	26.0 ± 2.0	31.0 ± 2.5	3/6 (33%)	06	1.0	2.0
7.	Cow urine alone	24.9 ± 1.6	29.8 ± 1.8	0/6	00	00	00

* Significance level among different groups at $p < 0.05$.

DISCUSSIONS

Now-a-days, chemoprevention is an important strategy to control the process of cancer induction. Therefore, there is a need for exploring medicinal plants or other natural agents that can work as chemopreventive agents. The present study demonstrates the chemopreventive potential of Cow urine on DMBA induced skin tumorigenesis in *Swiss albino* mice. Literature suggests that one sub-minimal dose of carcinogen initiates the process of carcinogenesis and the treatment with croton oil promotes them to visible tumor stage [13].

The present investigation exhibited the same with 100% tumor incidence and number of tumor yield as well as tumor burdon as 2.8 in carcinogen control group animals. This is perhaps due to the free radical oxidative stress that has been implicated in the pathogenesis of a wide variety of clinical disorders, resulting usually from deficient natural antioxidant efences as well as lipid peroxidation. On the other hand, animals of group which received similar treatment of DMBA and croton oil; when subjected to topical application of Cow urine, a significant reduction ($p < 0.05$) in tumor incidence, tumor yield and tumor burdon were recorded. The cumulative numbers of papillomas were found to be reduced in the Cow urine treated groups when compared to the carcinogen control group mice. The present findings also showed an increase in the percent inhibition of tumor multiplicity in the Cow urine treated groups in comparison to the control group.

The various properties of Cow urine was reported in the literature such as antigenotoxic and antioxidant properties in vitro [5], prevention of toxic effect of cadmium chloride in mice [6], the immunomodulatory effect in mice [7], to enhance cellular and humeral immune response [8]. It helps the lymphocytes to survive and to repair the damaged DNA and thus it is effective for cancer therapy [9]. Its protective effect on lymphocytes of birds undergoing apoptosis was reported [8]. It also prevented the pathogenic effects of free radicals through cow urine therapy. Beneficial effect of cow urine on serum biochemical profiles i.e total serum protein, glucose, calcium and cholesterol) of laying birds [7,11] was also reported. It was observed from our experiment that cow urine acts as an anticarcinogen in skin carcinogenesis assay. The treatment of cow urine with known carcinogens DMBA and croton oil has prevented the development of skin tumours. These reports are important because a lot of attention is being paid abot cow therapy.

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