# Anticancer effect of Taxus baccata and Indian cow urine distillate (CUD) on mice treated with Diethyl Nitrosamine – Pathomorphological study

Ankita Joshi, R S Chauhan

**Abstract** Cancer is a class of diseases in which a group of cells display the traits of uncontrolled growth, invasion and its spread through metastasis to the distant body places. Cancer is mainly caused by abnormalities in the genetic material of the transformed cell. The abnormalities may be due to the effect of carcinogen such as tobacco, smoke, radiation, and Chemicals like; DEN, aflatoxins and other nitroso compounds etc.Among various cancers, liver cancer is one of the most frequent that kill more than 600,000 people around the world every year. The present investigation was to study the effect of extracts of Taxus baccata alone and in combination with indigenous Cow Urine Distillate (CUD) in mice. Carcinogenicity was induced by diethyl nitrosamine (200µl/kg) (DEN). In a single dose DEN challenged animals were given extracts alone and in combination with CUD, daily and named them as test group. For control group no DEN and no treatment was given. Negative control was given only DEN, to check its carcinogenicity with control and test group. Tumors were developed in negative control animals, which died early but no mortality was observed in the test group. After six month the tissues from liver and kidneys were collected and histopathology was done to study the effect of carcinogen, plant extracts and their formulation with CUD. Microscopic examination of liver showed the presence of hyperplastic and anaplastic cells with the characteristic features of anaplasia including pleomorphism, hyperchromasia and presence of mitotic figure.

**Keywords:** Cancer, cow urine, diethyl nitrosamine, mice, Taxus baccata

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## INTRODUCTION

Cancer is the uncontrolled growth of malignant cells, which if left unchecked, can destroy organs or their functions. Cancer cells are able to divide more rapidly than normal cells and can displace normal neighboring cells. Cancer may affect people at any age, but risk tends to increase with age [1]. Present day cancer is one of the biggest health problems worldwide.Cancers are caused by abnormalities in the genetic material of the transformed cells [2]. These abnormalities may be due to the effects of carcinogens, such as tobacco smoke, radiation, chemicals, or infectious agents. Cancer-promoting genetic abnormalities may randomly occur through errors in DNA replication, or are inherited, and thus present in all cells from birth. Cancers are usually affected by complex interactions between carcinogens and the host's genome [3]. The alkylating agent DEN (diethylnitrosamine) is a well-studied liver carcinogen, when administered continuously to experimental animals produce a well-characterized cancer in liver [4].Cancer can be detected in a number of ways, including the presence of certain signs and symptoms, screening tests, or medical imaging. Once a possible cancer is detected, it is diagnosed by microscopic examination of a tissue sample. Cancer is usually treated with chemotherapy, radiation therapy and surgery [5].

There are various Complementary and Alternative Medicine (CAM) used in cancer patients' worldwide, namely herbal medicine, acupuncture, Ayurveda, biological agents, traditional Chinese medicines, meditation and yoga etc. The chemotherapy and radiation therapy are known to cause various serious side effects and many times become fatal to the patient. Besides, many herbs having anticancereffects *Taxus baccata* is known to contain taxane/taxol which exerts anticancer effect [6]. Similarly, indigenous cow urine has also been found to have anticancer, immunomodulatory and bio-enhancer effects in experimental animals [7].

Hence, considering all these points, the present study was planned to evaluate the anticancer effects of *Taxus baccata* and cow urine as single and in combination in mice treated with DEN through the histopathology of liver and kidney.

## Material and Methods

**Plant material:** Plant material was collected from *Taxus baccata* tree, which is commonly known as THUNER, from the premises of the Institute of Biotechnology,Patwadangar, Nainital, Uttarakhand. Plant materialwas washed 3 to 4 times by

tap water and 4 to 5 timesby distilled water. Then it was shade dried. Plant extracts were prepared by applying the standard methods with different solvents like Aqueous, ethanol, methanol, ether etc [8][9].

**Cow urine:** The urine from Badri cow was collected asper standard procedure directly from cows urinating in the early morning and by stimulating the vagina/vulva.The urine was then subjected to filter through muslin cloth, distilled and 50% distilled product was taken for *in-vivo* study.

**Experimental design:** The experiment was performed in mice maintained in the experimental animal house in Institute of Biotechnology, G.B. Pant University of Agriculture and Technology, Patwadangar, Nainital, Uttarakhand, India. A total of 89 animals were equallydivided into 11 groups. The mice were housed in clean polypropylene cages and fed ad-libitum withcommercially available feed and water. The experiment was carried out in accordance with the guidelines of the Institutional Animal Ethical Committee of G.B. Pant University.

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| 1. Control group | 9 mice |
| 2. Negative control (DEN treated mice) | 8 mice |
| 3. A (Aqueous extract of leaves of *Taxus baccata*) | 8 mice |
| 4. B (Ethanolic extract of leaves of *Taxus baccata*) | 8 mice |
| 5. G (Methanolic extract of bark of *Taxus baccata*) | 8 mice |
| 6. H (Ether extract of Bark of *Taxus baccata*) | 8 mice |
| 7. CUD (Cow Urine Distillate) | 8 mice |
| 8. CUD+A | 8 mice |
| 9. CUD+B | 8 mice |
| 10. CUD + G | 8 mice |
| 11. CUD+H | 8 mice |



Single dose of diethyl nitrosamine (DEN) @ 200 µl/kg body weight was given to each mice of negative control group and test groups. 500 ml of each extract was made by adding 20% of extract in 500ml of distilled water.The mice of 9 test groups were given different extracts of *Taxus baccata* alone and in combination with CUD (2ml/day/mice), daily p.o., from day 1 for 6 months; however,the mice of negative control group were maintained with normal feed and water.

After 6 month of treatment all the mice were sacrificed. The mice were euthanized at the end of the experiment i.e, after six month. Necropsy examination was performed as per standard procedure and gross lesions were recorded. The tissues were collected during port mortem examination of mice sacrificed at the end of experiment in formol saline. The tissues were processed as per standard procedure and sections were cut at 4-5 micron thickness and were stained with H and E [10].

## Results

In the end of experiment i.e., after completing six month, the experimental mice were euthanized for gross and microscopic examination of different organ.

**Necropy**

Necropsy examination of experimental mice showed the presence of single and/or multiple nodules in the dorsal and ventral surfaces of liver (Fig. 1). The size of tumor varied from few millimeters to several millimeter and even in some cases the tumor was diffused in whole of the organ leading to the enlargement and discoloration of the liver. In few cases the liver was enlarged and became pulpy. Nothing abnormal was detected in other organs on gross pathological examination. These nodules were present on liver of only in DEN treated mice. In the mice of other group, there were mild enlargement of liver and/or presence of tiny nodules were recorded (Fig.2).

## Histopathology

Microscopic examination of liver showed the presence of hyperplastic and anaplastic cells with the characteristic features of anaplasia including pleomorphism, hyperchromasia and presence of mitotic figures. At several places, the vaculation were also observed in the hepatic cells. At many places, there were apoptotic cells leading to the disintegration of the liver parenchymatous tissue (Fig. 3-6). Microscopic examination of lung revealed the presence of malignant cells in the blood vessels indicating metastasis of the tumor cells through blood. Lung parenchyma showed the infiltration of mononuclear cells and congestion at places (Fig. 7). Microscopic examination of kidney revealed the congestion and haemorage at places (Fig. 8), there was degeneration and necrosis in tubular epithelium.

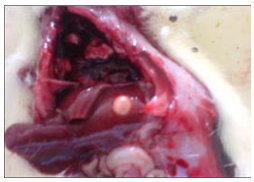

Fig 1:Necropsy examination of experimental mice.



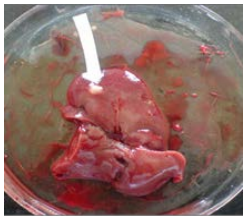

Fig 2:enlargement of liver and/or presence of tiny nodule.

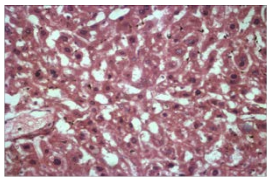

Fig 3: Photograph of liver showing presence of anaplasia, including pleomorphism, hyperchromasia, mitotic figures, apoptosis in transformed cells (400x)

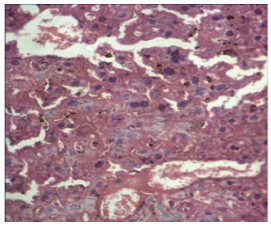

Fig 4: Photomicrograph of liver showing mitotic figure, anaplasia, congestion and apoptosis in some transformed cells(400x)

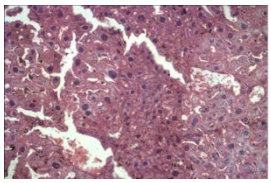

Fig 5: Photomicrograph of liver showing mitotic figure, anaplasia, congestion and apoptosis in some transformed cells (400x)

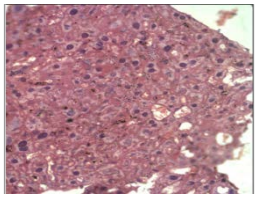

Fig 6: Photomicrograph of liver showing presence of mitotic figure, apoptosis in transformed cells (400x)

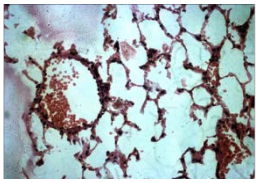

Fig 7: Photomicrograph of lung showing presence of tumour cells in blood vessel with mild congestion and infiltration of mononuclear cells in alveolar wall (400x)

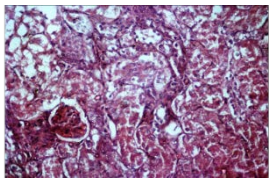

Fig 8: Photomicrograph of kidney showing necrosis of tubular epithelium (400x)



## DISCUSSION

Gross and microscopic examination of liver showed the lesions of malignancy, conferming the clinicopathological studies in DEN treated mice. The presence of tumor on liver in DEN treated mice and its absence or presence tiny size tumor in *Taxus baccata* and CUD treated mice further conform the occurrence of tumor due to DEN and its regression due to *Taxus baccata* and CUD. Cow urine possesses anti-cancer properties. Research works carried out by Go-Vigyan Anusandhan Kendra (Cow Science Research Center) at Nagpur revealed the beneficial properties of cow urine in the treatment of cancers. Further extensive research on cow urine therapy against fighting cancer carried out by Scientists of Central Institute of Medicinal and Aromatic Plants (CIMAP), CSIR Center at Lucknow, along with collaboration with Go-Vigyan Anusandhan Kendra, Nagpur confirmed this milestone achievement. Studies highlight the role of cow urine in curing cancers and that cow urine enhances the efficacy and potency of anti-cancer drugs. Recently, this significant achievement has been validated by the grant of U.S. Patent (No. 6896907) in the field of treatment of cancers [11].

Scientists have proved that the pesticides even at very low doses cause apoptosis (cell suicide) in lymphocytes of blood and tissues through fragmentation of DNA. Distilled cow urine protects DNA and repairs it rapidly as observed after damage due to pesticides [12]. It protects chromosomal aberrations by mitocycin in human leukocyte [13]. Cow urine helps the lymphocytes to survive and not to commit suicide (apoptosis). Kumar et al., (2004) [14] reported the prevention of pathogenic effect of free radicals through cow urine therapy. These radicals cause 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. By regular use of cow urine one can get the charm of a youth as it prevents the free radicals formation [15]. The presence of tumor cell in lung blood vessel was indicative of metastasis but they could not lodge in lung tissue due to the effect of *Taxus baccata* and CUD.

## CONCLUSION

In the present study, it has been observed that cancer can be produced in mice using DEN and treatment experiment were conducted accordingly *T. baccata* leaves and bark extracts alone and along with CUD showed good cytotoxicity, good recovery in clinico pathological parameters of affected mice during the experimental period. Out of various combinations *T. baccata* aqueous and ethanolic extract of leaf with CUD was found most promising anticancereous preparation. This preparation is having both the qualities of *Taxus baccata* and CUD and their synergistic or bioenhancer effects, combined together might be helpful in controlling the cancer leading to recovery. The findings of this study are substantial and preliminary in nature which further needs detailed *in-vivo* experiments in experimental animals and there after clinical trials in human volunteers following the standard guidelines, before being release of such products in the market.

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1. Corresponding author – Ankita joshi, joshi.ankita11@gmail.com
2. Secound Author – Prof. RS Chauhan, rs_chauhan123@rediffmail.com

[1]P hd Scholar of Venkateshwara University.

[2]G.B. Pant University of Agriculture and Technology, Pantnagar-263145,U.S.Nagar, Uttarakhand)