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CHEMOPREVENTIVE EFFECT OF MORCHELLA ESCULENTA AGAINST DMBA INDUCED SKIN PAPILLOMA IN MICE

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ABSTRACT

Chemoprevention is an important strategy to control the process of carcinogenesis. There has been a growing awareness in recent years that dietary non-nutrient compounds can have important effects as chemopreventive agents. Mushrooms have long been attracting a great deal of interest in many areas of foods and biopharmaceuticals and have a great potential for the production of useful bioactive metabolites and are prolific resource for drugs. *Morchella esculenta* (L) Pers. is an excellently edible and nutritious mushroom belonging to Ascomycotina division. The present study evaluated the inhibitory action of *Morchella esculenta* extract against DMBA induced and croton oil promoted skin pappilloma in swiss albino mice**.** The aqueous-ethanol extract of *Morchella esculenta* (1mg and 5mg) was given orally two weeks before the start of the experiment and applied topically 40 minutes before croton oil application. Average number of papilloma per mice, percent of animals with papilloma and tumor latency period were recorded. Significant reduction in number of papilloma per mice, percent of animals with papilloma and a marked increase in average latent period of tumor appearance were found after the treatment with the extract. The extract may have the immunotherapeutic property to inhibit growth of tumour cells and exert carcinostatic action. The antioxidant and antiinflammatory activity of the extract may also contribute to its anticancer property. Hence *M esculenta* mycelium possibly provides additive or synergistic effect in the prevention and treatment of cancer.

KEYWORDS: Chemoprevention, Carcinogenesis, mushroom, Morchella esculenta, skin papilloma.

INTRODUCTION

Cancer is one of the leading causes responsible for human death.[1] (Gao *et al*, 2005). Carcinogenesis, a multistage process that involves molecular and cellular alterations, largely consists of three separate, but closely linked stages—tumor initiation, promotion and progression.[2,3,4] Initiation is a rapid and irreversible process that involves a chain of extracellular and intracellular events. These include the initial uptake of or exposure to a carcinogenic agent, its distribution and transport to organs and tissues where metabolic activation and detoxification can occur, and the covalent interaction of reactive species with target-cell DNA, leading to genotoxic damage. In contrast to initiation, tumor promotion is recognized as a relatively lengthy and reversible process in which actively proliferating preneoplastic cells accumulate. Progression, the final stage of neoplastic transformation, involves the growth of a tumor with invasive and metastatic potential.

Skin cancer is mainly caused by various environmental carcinogens, inflammatory agents, ultraviolet (UV) irradiation and/or tumor promoters. Murine skin tumorigenesis is an excellent *in vivo* model to study the chemopreventive activity of natural/synthetic agents.[5] Tumor development can also be caused by multiple applications of croton oil, a tumor promoter, which is a slow and reversible process and leads to skin tumors in mouse by inducing hyperplasia and inflammatory responses.[6,7] 7, 12‑dimethylbenz[a] anthracene (DMBA), a carcinogen, initiates skin tumorigenesis which is a rapid and irreversible process. The croton oil is the most widely used distinguished promoting agent to understand the cellular and molecular alterations associated with promotion stage and also a well‑known model to understand the role of inflammation, generation of reactive oxygen species (ROS), and hyperplasia in cancer promotion.[8]

Cancer mortality nowadays remains unacceptably high despite immense advances in the understanding of the mechanisms of carcinogenesis, in bringing potent new drugs to the clinic and in treating several rare forms of cancer. Cancer chemotherapy represents a very promising strategy in the global effort of cancer prevention and treatment.[9] Conventional cancer chemotherapy is used to kill or disable tumor cells while

preserving the normal cells in the body by application of synthetic compounds.[10] These agents have a narrow margin of safety, and the therapy may fail due to drug resistance and dose limiting toxicities, which may severely affect the host normal cells.[1] Hence the use of natural products has been contemplated of significant value in the control of cancer and its eradication programme.[11] The enhancement or potentiation of host defense mechanisms also emerges as a possible means of inhibiting tumour growth without harming the host.

There is significant interest in the use of mushrooms and /or mushroom extracts as dietary supplements based on theories that they enhance immune function and promote health.[12] Extracts of many mushrooms used in traditional Chinese medicine and other folk medicine have been reported to be efficacious in the treatment of various diseases including many forms of cancer. The use of medicinal mushroom extracts in the fight against cancer is well documented in China, Japan, Korea, Russia and now increasingly in the USA.[13]

Recently dietary intervention is encouraging and emerging as an acceptable approach for controlling the cancer incidence worldwide.[14,15] Mushroom metabolites are increasingly being utilized to treat a wide variety of diseases, particularly as they can be added to the diet and used orally, without the need to go through phase-I/II/III trials as a synthetic drug, and they are considered as safe and useful for disease treatment.[16] Most mushroom derived preparations and substances find their use not as pharmaceuticals (real medicines) but as a novel class of dietary supplements (DS) or nutriceuticals.[17]

Morchella esculenta (L) Pers. is an excellently edible and nutritious morel mushroom belonging to Ascomycotina division. Mycelium of mushrooms obtained from pure cultures is an ideal choice for developing consistent and safe health care products. In our previous studies cultured mycelium of *M.esculenta* showed higher antioxidant and anti-inflammatory activity. Polysaccharides from *M.esculenta* were also reported to possess immunomodulatory property by activating macrophages.[18] Preventive roles of antioxidants on cancer development have been repeatedly suggested.[19] The major mechanism underlying such chemopreventive action has been supposed to be suppression of the formation of free radicals and other reactive oxygen species that are likely to be involved in both initiation and promotion steps of carcinogenesis.[20]

MATERIALS AND METHODS

Production of mushroom mycelium

Culture of *Morchella esculenta* obtained from the Microbial Type Culture Collection (MTCC 1795), Institute of Microbiology, Chandigarh, India, was employed for the studies. The fungus was grown in submerged culture on potato-dextrose broth (PDB) for the production of mycelia biomass. After ten days of growth in submerged culture on a shaker at 24–25°C, the fungal biomass was harvested, washed thoroughly and dried at 40–50°C.[21]

Preparation of extracts

The dried mycelia was powered and 100 g of powder was extracted with hot aqueous-ethyl alcohol (ethyl alcohol: water 50/50 v/v) for 8-10 h. The extract was concentrated at low temperature and solvent completely evaporated under vacuum. The residue thus obtained was used for the experiments.

Animals

Female Swiss albino mice of 6 weeks old weighing 25 ± 2 g were employed for anticarcinogenic studies. These animals were housed in polypropylene cages in the animal house at temperatures of 24 ± 30°C, supplemented with food (Hindustan Lever Ltd. India) and water ad libitum. The study protocol is approved by the Departmental Animal Ethical Committee.

Experimental Design for Skin Carcinogenesis

Female Swiss albino mice were shaved on their back using surgical clippers 2 days before the experiment. The animals were randomly allocated into 3 groups of 10 animals each. The skin tumor was initiated with a single topical application of 390 n mol of 7,12-dimethyl benz [a] anthracene (DMBA) in 200µl acetone.[22] One week after tumor initiation, the promotion was induced by topical application of 200 µl of freshly isolated croton oil, 5% in acetone, v/v twice weekly for 8 weeks to the same area.[23,24] The aqueous-ethanol extract (1mg and 5mg in 200 µl acetone/mouse) was applied topically 40 minutes before each croton oil application. The group treated with DMBA- Croton oil alone served as positive control. Average number of papilloma per mice, percent of animals with papilloma and tumor latency period were recorded.

Treatment Groups

Group I (DMBA+Croton Oil) : Animals of the Group I received a single topical application of 390 n mol of 7,12-dimethyl benz [a] anthracene (DMBA) in 200µl acetone and 200 µl of freshly isolated croton oil (5%) twice weekly for 8 weeks in the same area.

Group II (DMBA+Croton Oil+ 1mg *Morchella esculenta* extract): The animals of Group I received 1mg /kg body weight of the *M. esculenta* extract prior to the start of the experiment. These animals were applied topically a single dose of 390 n mol of 7, 12-dimethyl benz [a] anthracene (DMBA) in 200µl acetone and 200 µl of freshly isolated croton oil (5%) twice weekly for 8 weeks in the same area. The animals of this group was applied topically the aqueous-ethanol extract of *M. esculenta* (1mg 200 µl acetone/mouse) 40 minutes before each croton oil application.

Group III(DMBA+Croton Oil+ 1mg *Morchella esculenta* extract): The animals of Group I received 5

mg /kg body weight of the *M. esculenta* extract prior to the start of the experiment. These animals were applied topically a single dose of 390 n mol of 7, 12-dimethyl benz [a] anthracene (DMBA) in 200µl acetone and 200 µl of freshly isolated croton oil (5%) twice weekly for 8 weeks in the same area. The animals of this group was applied topically the aqueous-ethanol extract of *M.esculenta* (5mg 200 µl acetone/mouse) 40 minutes before each croton oil application.

RESULTS AND DISCUSSION

Effect of *Morchella esculenta* Extract on DMBA induced skin papilloma

The findings of the present study are depicted in Figure 1, 2 and 3. Treatment with *Morchella esculenta* extract inhibited skin papilloma initiated by DMBA and promoted by croton oil on mouse skin significantly. The group of animals applied with croton oil and DMBA alone showed 80% tumor incidence after 18 weeks of DMBA treatment. Topical application of extract 1mg showed 60% and 5mg showed 40% tumor incidence at 18 weeks after DMBA application (Fig 1). The average number of tumor (1mm diameter) per animal in the control group which received DMBA + Croton oil alone was 3.42 at 18th week after DMBA application, while in the 1mg and 5mg extract treated groups, the average number of tumor per animal was 1.77 and 1.5 respectively (Fig 2). The tumor latency period was significantly increased with *M.esculenta* extract treatment. In the control group tumor latency period was 76.16 days, while in the 1 and 5mg extract treated group tumor latency period was increased to 95.44 and 98 days respectively (Fig 3).

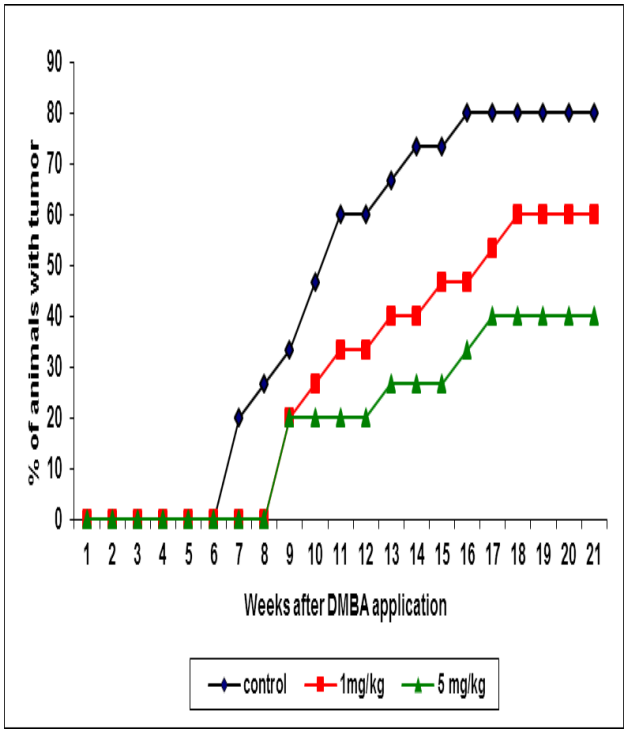

Fig .1 Effect of *M.esculenta* mycelium extract on % of animals with tumor
Values are mean ± S.D, n=10

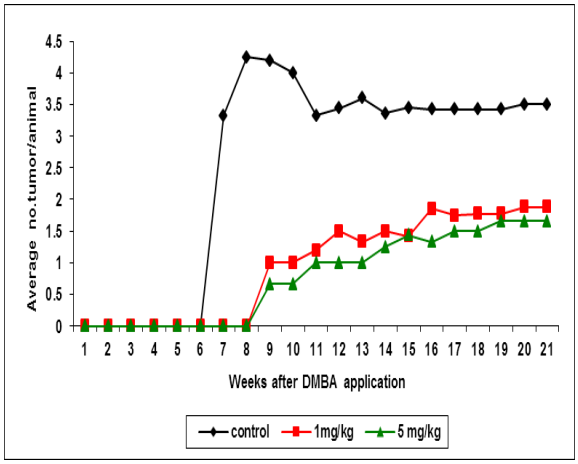

Fig .2 Effect of *M.esculenta* mycelium extract on average no: of mice with tumor
Values are mean ± S.D, n=10

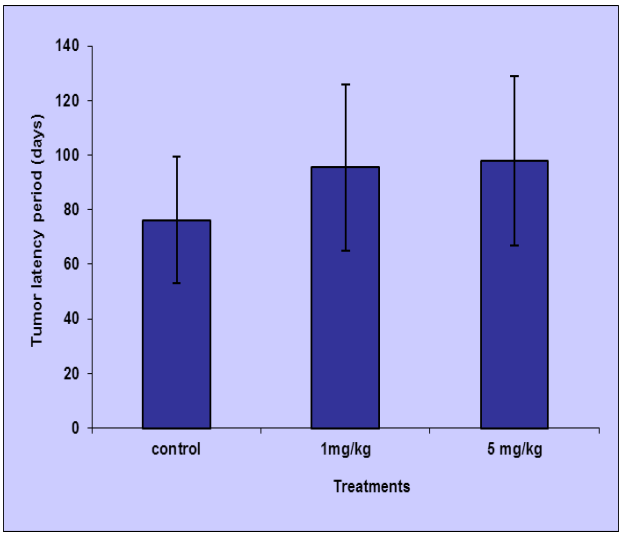

Fig. 3 Effect of *M.esculenta* mycelium extract on Tumor latency period
Values are mean ± S.D, n=10

The battle against cancer has been waged for several decades without resounding curative success from the use of chemotherapy or radiotherapy in most common solid tumors.[25] (Abel, 1992). Much of the present day research directed against active malignancy has shifted toward identification of strategies affecting the growth rate or apoptosis of such cells so that life with cancer can be greatly extended without the deleterious effects of the more aggressive therapies.[26] The large majority of malignancies are attributable to dietary and lifestyle factors. In addition there are many environmental chemical factors suspected of playing a sizable role in the occurrence of malignancies.[27] A good number of cancer causing chemicals are manmade and used either as industrial agents, pesticides and pharmaceuticals, chemicals or food additives.[28]

Many chemical compounds are carcinogenic only after the metabolic activation. 7, 12- dimethylbenz [a] anthracene (DMBA) a potent skin and breast carcinogen,

belonging to polycyclic aromatic hydrocarbons (PAH) also need metabolic activation for its carcinogenic property. Most of the metabolically activated PAHs are mutagenic to DNA.[29] Exposure to PAHs increases the expression of enzymes belonging to CYPIA and 1B subfamilies. They generate genotoxic epoxide metabolites of the parent hydrocarbon that can bind to DNA forming adducts.[30] These adduct if not repaired can cause specific mutation leading to cellular transformation. Therefore the activation and expression of carcinogen activating enzymes are key components in chemically induced carcinogenesis. 12-O-tetradecanoylphorbol-13 acetate (TPA) is a tumor promoter isolated from seed oil of *Croton tiglium* and has been extensively studied in DMBA induced mouse skin tumor model.

Experimental results from DMBA induced skin papilloma indicate that the application of the extract before each application of croton oil significantly reduces the tumor incidence and also the number of animals with tumor as compared to control group. The extract also inhibited the croton oil mediated skin inflammation. Our previous studies well revealed the antioxidant and anti-inflammatory activity of the extract. Polysaccharide isolated from the fruiting bodies of *M.esculenta* has been reported to exhibit immunostimulatory activity.[18] Although a complete answer to the antitumor mechanism of mushroom extracts, polysaccharides or polysaccharide-protein complexes is not yet available, they are generally considered as a kind of biological response modifiers which are able to restore or enhance various immune responses *in vivo* and *in vitro*. The extract may have the immunotherapeutic property to inhibit growth of tumour cells and exert carcinostatic action. The significant antioxidant and anti-inflammatory activity of the extract may also contribute to its significant antitumor and anticancer property. Hence morel mushroom mycelium possibly provides additive or synergistic effect in the prevention and treatment of cancer.

CONCLUSION

From the present study, it is evident that *Morchella esculenta* mycelium is a source of many anti-carcinogenic agents and antioxidants, which may be useful for the prevention of chemical induced skin cancer in mice. This work demands further study to evaluate the exact mechanism of chemoprevention offered by *Morchella esculenta* constituents as well as its possible chemo preventive efficacy against other types of tumors in various models.

REFERENCES

1. Gao Y, Gao, H, Chan E, Tang W, *et al.* Antitumor activity and underlying mechanisms of Ganopoly, the refined polysaccharide isolated extracted from *Ganoderma lucidum* in mice. Immunol Invest, 2005; (34): 171-198.
2. Surh YJ. Cancer chemoprevention with dietary phytochemicals. *Nature Rev Cancer*, 2003; (3): 768-80.
3. Moolgavkar SH. The multistage theory of carcinogenesisand the age distribution of cancer in man. *J Natl Cancer Inst*, 1978; (61): 49-52.
4. Armitage P, Doll R. A two-stage theory of carcinogenesis in relation to the age distribution of human cancer. *Br JCancer*, 1957; (11): 161-9.
5. Afaq F, Saleem M, Krueger CG, Reed JD, Mukhtar H. Anthocyanin and hydrolyzable tannin‐rich pomegranate fruit extract modulates MAPK and NF‐κB pathways and inhibits skin tumorigenesis in CD‐1mice. Int J Cancer 2005; (113): 423‐33.
6. DiGiovanni J. Modification of multistage skin carcinogenesis in mice. Prog Exp Tumor Res, 1991; (33): 192‐229.
7. Dibyajyoti S, Milan H. An ontological design: Two stage mouse skin carcinogenesis induced by DMBA and promoted by croton oil. Asian J Res Pharm Sci, 2012; (2): 1‐3.
8. Ha HY, Kim Y, Ryoo ZY, Kim TY. Inhibition of the TPA induced inflammation and hyperplasia by EC–SOD. Biochem Biophys Res Commun, 2006; (348): 450‐8.
9. Karamouzis MV. The status of chemoprevntion in the current cancer therapeutic armamentarium. J. BUON, 2004; 9(4): 363-372.
10. Fidler IJ and Ellis LM. Chemotherapeutic drugs-more really is not better. Nat Med, 2000; 6(5): 500-502.
11. Suffness M and Pezzuto JM. Assays related to cancer drug discovery. In: Methods in Plant Biochemistry, Academic Press, New York. 1991,6, pp 72-98.
12. Borchers AT, Keen LC, Gershwin EM. Mushrooms, Tumors and Immunity : An Update. Exp Biol Med (Maywood), 2004; (229): 393-406.
13. Mizuno T, Sakai T, Chihara G. Health Foods and medicinal usages of mushrooms. Food Rev Int, 1995; (11): 69-81.
14. Sporn MB, and Newton DL. Chemoprevention of cancer with retinoids. Fed Proc, 1979; (38): 2528-2534.
15. Kellof GJ. Perspective on cancer chemoprevention research and drug development. Adv in Can Res, 2000; (78): 199–334.
16. Lull C, Wichers HJ, Savelkoul FJ. Antiinflammatory and immunomodulating properties of fungal metabolites. Mediators. Inflamm, 2005; (2): 63-80.
17. Wasser SP, Nevo E, Sokolov D., ReshetnikovS, Timor-Tismenetsky M. Dietary supplements from medicinal mushrooms: diversity of types and variety of regulators. Int. J. Med. Mushr, 2000; 2(1): 1-19.
18. Duncan CJ, Pugh N, Pasco DS, Ross SA. Isolation of a galactomannan that enhances macrophage activation from the edible fungus *Morchella esculenta*. J Agric Food Chem, 2002; (50): 5681-568.

19. Byers T and Guerrero N. Epidemiologic evidence for vitamin C and vitamin E in cancer prevention. Am. J. Clin. Nutr, 1995; 62(6): 1385-1392.
20. Gromadzinska J and Wasowicz W. The role of reactive oxygen species in the development of malignancies. Int. J. Occup. Med. Environ. Health, 2000; 13(2): 233–245.
21. Janardhanan KK, Kaul TN, Husain A. Use of vegetable waste for the production of fungal protein from *Morchella* species. J Food Sci Technol, 1970; (7): 197–199.
22. Mimura A, Takebayashi K, Niwano, M, Takahara T, Osawa T, and Tokuda H. Oxidative and anticancer components produced by cell culture of sesame. In: Food Phytochemicals for cancer practice II. Teas, Spices and Herbs. Ho, C.T, Osawa T, Huang MT and Resen, RT, (Eds). Americal Clinical Society, Washington, D.C: 1994; 281-294.
23. Verma, AK, and Boutwell RK. Effects of dose and duration of treatment withtumor promoting agent, 12-O-tetradecanoyl-phorbol-13-acetate on mouse skin. Carcinogenesis, 1980; (1): 271-276.
24. Diwan BA, Ward JM, Henneman J, Wenk ML. Effect of short term exposure to the tumor promoter, 12-O-tetradecanoylphorbol-13-acetate on skin carcinogenesis in SENCAR mice. Cancer Lett, 1985; 26(2): 177-184.
25. Abel U. Chemotherapy of advanced epithelial cancer-a critical review. Biomed. Pharmacother, 1992; 46(10): 439-452.
26. Lamson DW, and Brignall MS. Natural agents in the prevention of cancer, part I: Human Chemoprevention trials. Alt. Med. Rev, 2001; 6(2): 7-19.
27. Crinnion WJ. Environmental medicine, part one: the human burden of environmental toxins and their common health effects. Altern. Med. Rev, 2000; (5): 52-63.
28. Ajith TA, Harikumar KB, ThasnaH, Sabu MC, Babitha NV. Proapoptotic and antitumor activities of the HMG-CoA reductase inhbitor, lovastatin, against Dalton's Lymphoma Ascites tumor in mice. Clin. Chim. Acta, 2006; (366): 322-328.
29. Miller EC. Some current perspective on chemical carcinogenesis in humans and experimental animals. Presidential address. Can. Res, 1978; (3)8: 1479-1496.
30. Gilbert, S.F. 1994. In: Developmental Biology. Sunderland, M.A: Sinauer Associates Inc.