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Prevention of mammary adenocarcinoma and skin tumour by *Ganoderma lucidum*, a medicinal mushroom occurring in South India

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***Ganoderma lucidum* (Fr.) P. Karst. is considered as a panacea in Chinese medicine. This mushroom has been reported to have a number of novel biological activities. Our previous investigations have demonstrated the antioxidant, anti-inflammatory and anti-tumour activities of aqueous-methanolic extract of *G. lucidum* occurring in South India. We extended our studies to evaluate the effect of this mushroom extract on the prevention of mammary adenocarcinoma in rats and skin tumour in mice. Mammary tumours were induced by oral administration of 7,12-dimethyl benz[*a*]anthracene (DMBA) in female Sprague Dawley rats. Skin tumours were induced by topical application of DMBA and promoted by croton oil on Balb/c mice. The experimental results indicated that *G. lucidum* showed significant tumour reducing activity against DMBA induced mammary and skin tumours in a dose-dependent manner. The administration of the mushroom extract showed profound effect on tumour induction, tumour latency period and tumour proliferation of both mammary tumour as well as skin tumours. The results thus reveal that the aqueous-mehanolic extract of *G. lucidum* possessed significant protective effect against DMBA induced mammary and skin tumours. Findings suggest the potential therapeutic use of *G. lucidum* in cancer chemoprevention.**

Keywords: *Ganoderma lucidum*, mammary adenocarcinoma, medicinal mushroom, skin tumour.

BREAST cancer is one of the most frequent malignancies among women and the incidence is increasing at an alarming rate. It is the major cause of cancer deaths in women worldwide, both in developed and developing countries[1,2]. Hope for treating cancer lies in four modules, surgery, radiation, chemotherapy and a combination of all the three[3]. Despite abundant information on the etiopathogenesis and early detection, effective therapeutic modalities for patients with advanced stages of the disease are still needed. Adjuvant therapy after ablative surgery is effective only when the tumour is detected early. The role of polycyclic aromatic hydrocarbons (PAH) is clearly implicated in the process of carcinogenesis especially 7,12-dimethylbenz[*a*]anthracene (DMBA), which is one of the most potent skin and breast carcinogens known. Most of the metabolically activated PAHs are mutagenic to DNA[4]. 12-*O*-tetradecanoylphorbol-13-acetate (TPA) is a tumour promoter isolated from seed oil of *Croton tiglium* and has been extensively studied in DMBA-induced mouse skin tumour model. Inflammation and free radicals have been associated with cancer in various tissues including skin tumour, bladder, stomach and colon. The experimental evidence strongly suggests the role of free radical mediated tumour promotion in phorbol ester promoted papilloma on the skin[5]. Application of croton oil has been shown to reduce antioxidant enzymes in both epidermal and inflammatory cells[6]. Inhibition of ROI (reactive oxygen intermediates) generation can serve as an important system for the identification of agents that can inhibit oxidative DNA damage as well as tumour promotion.

Several nonnutritive phytochemicals found in natural products associated with pharmacological attributes reveal that they inhibit/delay and or reverse cancer

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evoked by either environmental insults and/or lifestyle[7]. A number of these chemopreventive agents act at the initiation, promotion or progression stages conceptually associated with the ontogeny of multistage carcinogenesis.

Mushrooms have been valued throughout the world as both food and medicine for thousands of years. They represent a major and as yet largely untapped source of potent pharmaceutical products. In Chinese folklore, fruiting bodies of *Ganoderma lucidum* (Fr.) P. Karst., a highly ranked oriental traditional medicine, have been regarded as a panacea for all types of diseases[8]. Investigations carried out in our laboratory showed that *G. lucidum* occurring in South India possessed significant antioxidant, antitumour, anti-inflammatory and antinociceptive properties[9,10]. We examined the protective effects of *G. lucidum* extract against mammary adenocarcinoma and skin tumour. DMBA induced rat mammary tumour and DMBA-initiated and croton oil promoted mouse skin tumour were employed as experimental models. The results of the investigations are reported in this communication.

Swiss albino mice and Sprague Dawley rats were purchased from Small Animal Breeding Centre, Kerala Agricultural University, Mannuthy, Thrissur. The animals were kept for a week under environmentally controlled conditions with free access to standard food (Lipton, India) and water. The animal experiments were carried out according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the approval of Institutional Animal Ethics Committee.

Sporocarps of *G. lucidum* (Figure 1) were collected from the outskirts of Thrissur, Kerala. Type specimen was deposited in the Botany Laboratory Herbarium, Centre for Advanced Studies in Botany, University of Madras (HERB.MUBL, 3175). The sporocarps were cut into small pieces, dried at 40–50°C for 48 h and powdered. Two hundred gram samples of the powdered materials was extracted with petroleum ether. The defatted materials were air dried, then extracted with 70% methanol for 8–10 h[11]. The extracts were pooled and solvents completely evaporated at 40°C using a rotary vacuum evaporator and then lyophilized. The residue thus obtained was designated as aqueous methanol extract (4%). The extract was dissolved in distilled water and used for the experiments.

DMBA was purchased from Sigma (St. Louis, USA) and all other chemicals used in the study were of analytical grade. Croton oil was extracted from seeds of *C. tiglium* using petroleum ether.

For determining the effect of the extract on mammary tumour, female Sprague Dawley rats (40–50 days old) (140 g) were divided into 3 groups of 6 animals each and the treatment schedule was followed as mentioned here[12].

Group I: 10 mg DMBA per animal in olive oil was administered orally by gavage once a week for 3 weeks.

Group II: DMBA was administered as in group I and *G. lucidum* extract (500 mg/kg) was administered orally 24 h after the first administration of DMBA and continued once daily for 3 weeks.

Group III: DMBA was administered as in group I and *G. lucidum* extract (1000 mg/kg) was administered as in group II.

The rats were palpated for mammary tumours once a week, starting from 4 weeks after administration of DMBA and extract. Average number of tumours per tumour-bearing rat, percentage of animals with tumour and tumour latency period were recorded for a period of 17 weeks[12]. The animals were sacrificed under anaesthesia, tumours extirpated and weighed. Percentage of inhibition was calculated by the formula $(1 - B/A) \times 100$, where A is the average tumour weight of the control group and B is that of the treated group[13]. Histopathological

Figure 1. Sporocarp of *Ganoderma lucidum* growing on a tree trunk.

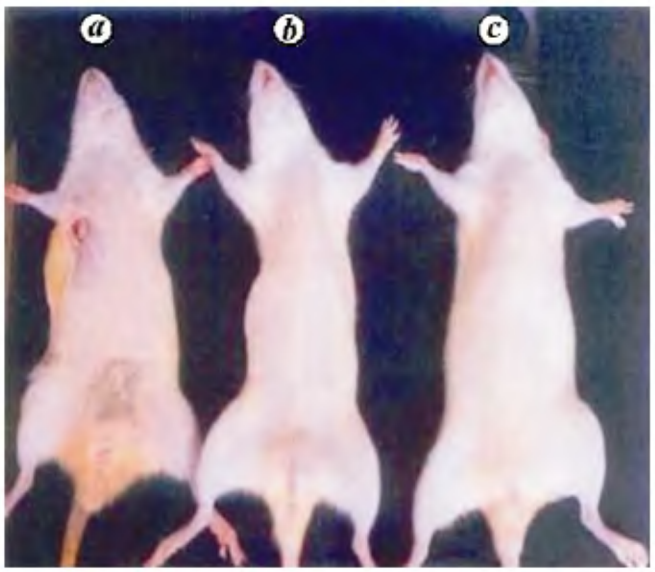

Figure 2. Mammary tumours produced in rats 12 weeks after DMBA administration. ***a***, Rat bearing mammary tumours induced by DMBA; ***b***, Mammary tumour bearing rat treated with *G. lucidum* extract (1000 mg/kg b.wt.); ***c***, Normal.

examination of the mammary tissues of animals of all experimental groups was carried out.

For determining the effect of the extract on skin tumour, backs of 40 female Balb/c mice (20–25 g) were shaved using surgical clippers, 2 days before the experiment. Animals with complete hair growth arrest were divided into 3 groups of eight animals each (Group-1: DMBA + croton oil (control), Group-2: DMBA + croton oil + 2 mg extract, Group-3: DMBA + croton oil + 10 mg extract). Skin tumour was initiated with a single topical application of 390 nmol of DMBA in 200 µl acetone[14]. One week after tumour initiation, the promotion was induced by topical application of 200 µl of freshly isolated croton oil (10% in acetone, v/v) twice weekly for 8 weeks on the same area[15]. Methanolic extract of *G. lucidum* (2 mg or 10 mg in 200 µl acetone/mouse) was applied topically 40 min prior to each croton oil application. The group treated with DMBA and croton oil served as positive control. Skin tumour formation was recorded weekly in each experimental group for 20 weeks. Average number of tumour per tumour bearing-mouse, percentage of animals with tumour and tumour latency period were recorded.

Preliminary chemical analysis of the extract was carried out to determine its major chemical components. The extract was tested with anthrone reagent[16] and with phenol–sulphuric acid reagent[17] for detecting polysaccharide component. Thin layer chromatographic (TLC) analysis of the extract was carried out on silica gel G using chloroform : methanol (90 : 10), chloroform : methanol : water (30 : 4 : 1) or ethyl acetate : methanol : water (100 : 13.5 : 10) as solvent systems. TLC plates were sprayed with vanillin–sulphuric acid reagent, alcoholic ferric chloride or anisaldehyde–sulphuric acid reagent[18]. The extract was also analysed by High Performance Thin Layer Chromatography (HPTLC) using CAMAG, HPTLC system with LINOMAT sample applicator. Ten mg of the extract was dissolved in 10 ml aqueous methanol and used for analysis. The sample (5 µl) was applied as bands using microsyringe on precoated silica gel 60 F254 plates (E. Merck). The plates after sample application were developed in twin trough chambers. Chloroform–methanol–water (30 : 4 : 1) was used as solvent system. The plates were air dried after development and scanned under UV (254 nm) or sprayed with vanillin sulphuric acid reagent and then scanned. Camag TLC scanner was used for scanning. HPTLC profile was obtained with Desaga Video Documentation Unit.

Experimental data are expressed as mean ± SD. One way analysis of variance (ANOVA) was applied for expressing the significant difference between groups. *P* value less than 0.05 was considered as significant.

All the surviving animals in the group treated with DMBA alone had mammary tumours (Figure 2). An average of 2 mammary tumours per animal were observed in the DMBA-alone treated group, after 12 weeks and 4 tumours after 17 weeks of DMBA administration (Figure 3), whereas animals treated with *G. lucidum* extract (1000 mg/kg) showed an average of one mammary tumour per animal and animals treated with low dose (500 mg/kg) of the extract showed an average of two tumours per animal respectively (Figure 3). DMBA-alone treatment induced 100% tumour incidence whereas in animals treated with *G. lucidum* extract (1000 mg/kg), the percentage of tumour incidence was 33.33% and animals treated with lower dose (500 mg/kg) the tumour incidence was 50% in 13th week after DMBA administration. This showed a dose-dependent decrease in tumour incidence (Figure 4). First mammary tumour was observed after 73 days in the group of animals administered with DMBA alone and in the group of animals treated with *G. lucidum* extract (1000 mg/kg) plus DMBA, the first tumour appeared only after 98 days. Treatment with the extract significantly inhibited tumour growth. This was evident from the tumour weight (Figure 5).

Histopathological examination of the mammary tissue showed that DMBA induced undifferentiated carcinoma

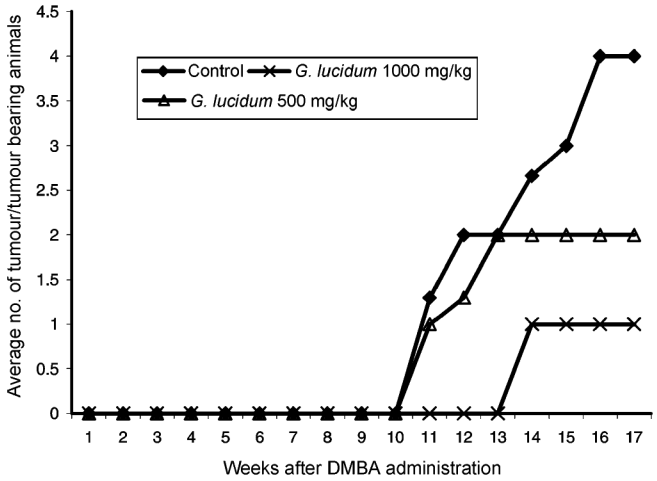

Figure 3. Effect of *G. lucidum* extract on DMBA induced mammary tumour: cumulative number of tumours per animal.

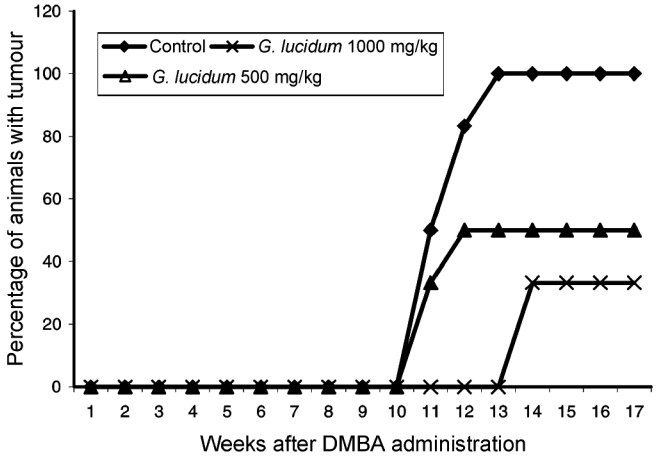

Figure 4. Effect of *G. lucidum* extract on DMBA induced mammary tumour: percentage of animals with tumour.

with marked nuclear pleomorphism and high mitotic index. Treatment with the mushroom extract could ameliorate the histopathological alterations to a significant extent (Figure 6). Animals treated with DMBA alone showed typical hyperplasia and invasive ductal carcinoma while the extract treatment was found to inhibit the necrosis of epithelial cells.

Topical application of *G. lucidum* extract inhibited mouse skin tumour initiated by DMBA and promoted by croton oil (Figure 7). The tumour latency period in the DMBA + croton oil and *G. lucidum* extract (10 mg) + DMBA + croton oil applied group of animals was 35 and 56 days respectively. The tumour incidence in the group of animals treated with DMBA and croton oil was 87.5%, 13 weeks after the application of DMBA. Average number of tumours per animal in the control group was 4 at 12 weeks and 7 at 16 weeks after application of croton oil. However, the average number of tumours per animal in the 2 mg and 10 mg *G. lucidum* extract treated group of animals was 2 and 1.6 respectively (Figure 8). Application of croton oil significantly promoted tumour development. However, the application of the extract prior to croton oil application reduced the percentage of tumour incidence to 37.5% in 13th week (Figure 9).

Phytochemical analysis of the extract showed that it reacted with the anthrone reagent and also with phenol–sulphuric acid reagent forming deep colour indicating polysaccharide as one of the major components. TLC analysis showed a number of spots. The major compound was detected by anisaldehyde–sulphuric reagent indicating it to be a terpenoid. A number of minor spots were also detected showing the presence of compounds that are in traces. HPTLC analysis also confirmed this observation. The phytochemical analysis, thus indicated that the major components of the extract were polysaccharides and terpenes (Figure 10).

Breast cancer is the most commonly occurring neoplastic disease in women worldwide and the increasing trend of this malignancy in urban population is a matter of great concern[19]. Results of the present investigation indicate that aqueous methanol extract of *G. lucidum* possessed profound protective effect against DMBA-induced mammary adenocarcinoma in rats. Administration of the extract at concentrations of 500 and 1000 mg/kg significantly reduced the number of tumour bearing animals in a dose dependent manner. The extract also could prevent the tumour growth and development in a dose dependent

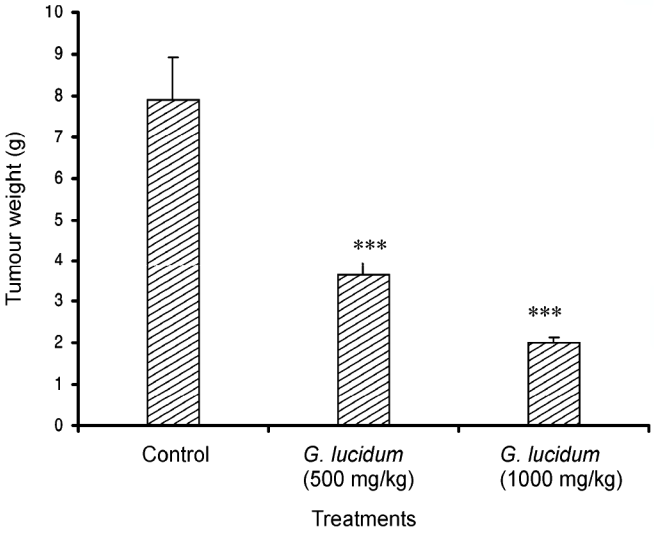

Figure 5. Effect of *G. lucidum* extract on DMBA-induced mammary tumour in rats – effect of tumour growth inhibition. Values are mean ± SD. ***$P < 0.001$ (Dunnett's test) significantly different from the control.

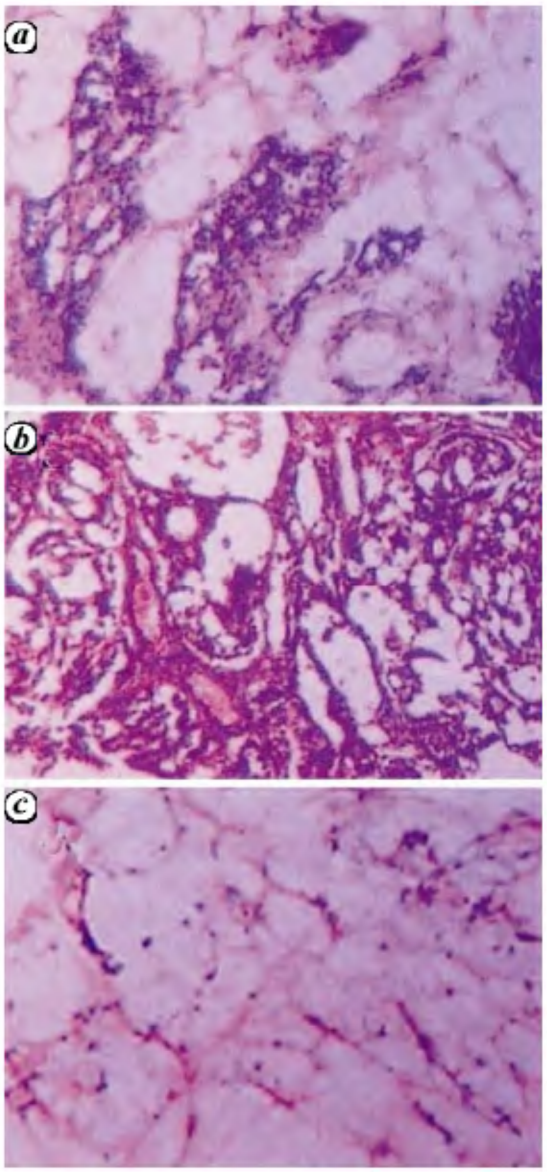

Figure 6. Histopathological evaluation of the effect of *G. lucidum* extract on pathological alterations in mammary tissues. ***a***, Mammary tissue from normal rat; ***b***, Mammary tissue from rat bearing tumour induced by DMBA; ***c***, Mammary tissue from rat bearing tumour treated with *G. lucidum* extract (1000 mg/kg b.wt.).

manner. Estrogens are clearly related to the pathological process of breast cancer. Oxidative catabolism of estrogen generates reactive free radicals that may cause oxidative damage. Reactive oxygen species (ROS) induce lipid peroxidation and generate toxic aldehydes such as malondialdehyde (MDA) that cause tumour promotion[20]. Our previous studies have demonstrated that extracts of *G. lucidum* possessed significant antioxidant activity[9,21].

In recent years, cancer chemoprevention by biologically active dietary or nondietary supplements has generated immense interest in view of their putative role in attenuating the risk of developing cancer[22]. The present study demonstrates the chemopreventive activity of *G. lucidum* extract. The administration of mushroom extract for the prevention of tumour formation in both DMBA initiated mammary tumours and skin tumour promoted by

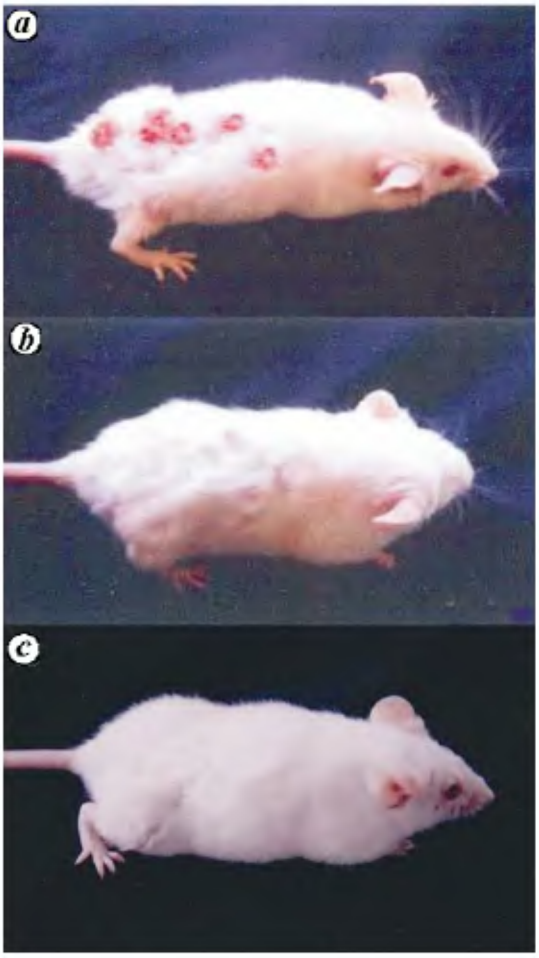

Figure 7. Mouse skin tumour induced by tropical administration of DMBA and promoted by croton oil – 15 weeks after DMBA application. ***a***, Mouse bearing skin tumours induced by DMBA; ***b***, Mouse bearing skin tumour treated with *G. lucidum* extract (10 mg); ***c***, Normal.

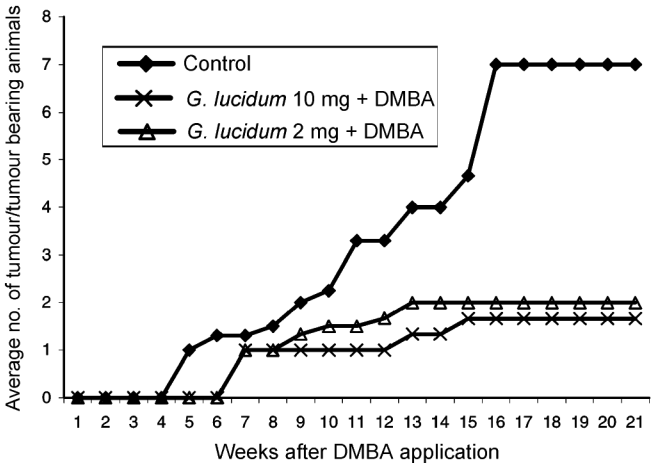

Figure 8. Effect of *G. lucidum* extract on mouse skin tumour induced by DMBA and promoted by croton oil: tumour incidence.

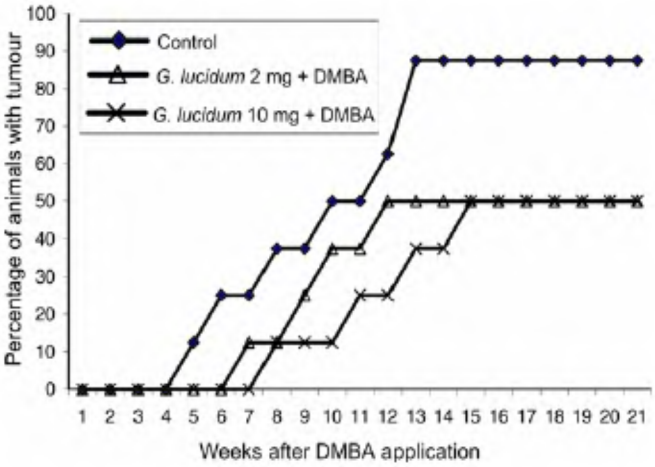

Figure 9. Effect of *G. lucidum* extract on mouse skin tumour induced by DMBA and promoted by croton oil: tumour bearing animals.

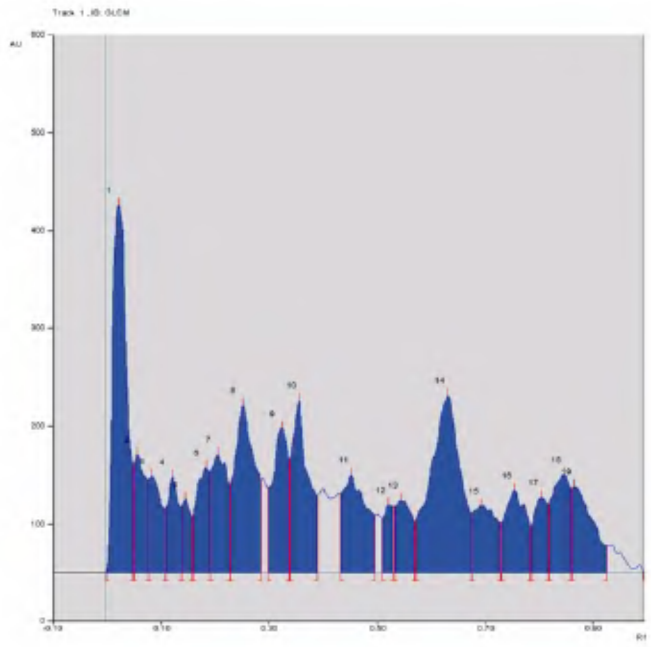

Figure 10. HPTLC profile of *G. lucidum* extract. The major peaks are of polysaccharides and terpenes.

croton oil opens up new approaches in chemoprevention. Treatment of mouse skin with TPA present in croton oil induced the production of free radicals in keratinocytes.

Phytochemical analysis of the methanolic extract of *G. lucidum* indicates that the major chemical components of the extract are polysaccharides and terpenoids. Several previous studies have demonstrated that the major chemical components of the fruiting bodies of this mushroom are polysaccharides and triterpenes[23,24].

The important mechanism of action of many chemopreventive agents is through their ability to modulate metabolic activation of a procarcinogen or by increasing the detoxification of reactive metabolites or scavenging the free radicals produced during the process of carcinogenesis. Polycyclic aromatic hydrocarbons must be metabolically activated to electrophilic intermediates, which can bind to DNA and exert their carcinogenic effects. Current experimental evidence indicates that metabolic activation of DMBA occurs primarily through the formation of 3,4-diol-1,2 epoxide[25]. In polycyclic aromatic hydrocarbon-induced tumourigenicity, oxidative phase I biotransformation results in highly reactive diolepoxides that form a covalent adduct with DNA. The DNA adduct formation is a prerequisite for carcinogenesis as shown for majority of known carcinogens[26]. The ability of chemical carcinogens to form DNA adducts can cause mutations and induction of tumourgenesis[27]. Reports indicate that the level of PAH-DNA adducts is related to the level of CYP1A1 expression[28]. Furthermore, mushroom extract also induces phase II detoxification enzymes as evident from our earlier studies[29]. Thus, the observed chemopreventive activity of *G. lucidum* extract might be either at the level of inhibition of procarcinogen activation, leading to reduced bioactivated DMBA metabolites or by increased expression of phase II detoxification enzymes. The experimental results indicate that aqueous methanolic extract of *G. lucidum* possesses significant protective effect against DMBA-induced mammary tumour and antipromotional effect against skin tumour. *G. lucidum* with its antitumour activity and the *Ganoderma* polysaccharides with a broad spectrum of immunemodulating activities may represent a novel immunotherapeutic agent in cancer therapy[30]. The findings, thus suggest the therapeutic potential of *G. lucidum* mushroom in cancer prevention.

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Discovery of minamiite from the Deccan Volcanic Province, India: implications for Martian surface exploration

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Discovery of minamiite, a Ca-bearing hydrous sulphate mineral, is reported for the first time in India from the Deccan Volcanic Province at Matanumadh (Kachchh, Gujarat), western India. Minamiite was identified by X-ray diffraction and chemical analyses. The hydrous sulphates at Matanumadh occur associated with Paleocene sediments as soft powdery deposits and consist dominantly of minamiite [(Na,K,Ca)$Al_3(SO_4)_2(OH)_6$] and natroalunite [$NaAl_3(SO_4)_2(OH)_6$] with traces of alunite and kaolinite. The geological setting, mineralogy and geochemistry suggest that the sulphate layers at Matanumadh were produced via solfataric alteration of volcanic ash. Hydrous sulphates are abundant on the planet Mars but not common on Earth because they form under extreme conditions (low pH and high Eh). Results of our study suggest that the Deccan Volcanic Province with its gigantic flood basalts of thermal plume origin, craters of both volcanic and impact origin, and hydrous sulphates of secondary origin can serve as a potential Earth analogue for the Mars and the Martian conditions. This may help us better understand the geology of the planet Mars and compare the geologic processes on Mars and the Earth.

Keywords: Deccan Volcanic Province, Earth analogue, Mars, fumarole activity, hydrous sulphates, minamiite.

THE discovery of liquid water-related sulphates in the Meridiani Planum region of the planet Mars has stimulated interest in studying the Martian analogues on Earth in order to elucidate the mineralogical and chemical makeup of the Martian surface, and to get insights into the processes that may have operated on Mars[1–12]. In the international arena, it is widely recognized that interpretations of Mars must begin by using the Earth as a reference, because Earth analogues can provide ground truth to constrain interpretations on the geological history of Mars. Accordingly, several sites such as Rio Tinto and Jaroso Ravine (Spain), Dry Valleys (Antarctica), Devon Island (Canada), Valley of Ten Thousand Smokes (USA), and Payun Matru Volcanic complex (Argentina), among others, are under investigation the world over.

In India, the Deccan Volcanic Province (DVP) spread over the large tracts of west-central parts of the country[13] could be a potential analogue for Mars, because of the similarity of its characteristic flood basalts, craters of both impact and volcanic origin[14], and extensive hydrous sulphate layers to those observed on Mars. The Deccan volcanic flows represent some of the largest sub-aerial lava flows on the Earth surface, and show a multiplicity of flow surface morphologies linked to different lava types and related emplacement mechanisms. Interestingly, the Deccan basaltic volcanism on Earth as well as the volcanoes of the Tharsis region, in general and the Olympus Mons in particular, on Mars were produced by the thermal plume process (hotspot). However, the mammoth size of volcanoes on the Mars compared to those on the Earth is considered to be due to the absence of active plate tectonics on the Mars[15]. Therefore, the DVP can represent an outstanding analogue of several Martian flows. In addition, the understanding of propagation processes of widespread Deccan basalt flows can give important clues in the comprehension of emplacement mechanisms of the long flows on Mars.

The Deccan basalts, by virtue of their thickness, spatial extent and eruption timing coinciding with the Cretaceous–Tertiary (K–T) boundary event have been studied extensively for petrological, geochemical, biotic and paleomagnetic aspects to understand their impact on climate at the K–T boundary[16–23]. However, very little is known about the nature of rock-alteration assemblages formed due to aqueous processes that operated during the gigantic volcanic activity. Alteration phases are important recorders of atmosphere–fluid–rock interactions, specific surface processes, environments and fluid compositions during the volcanism, and thus, can provide a template

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