Research Article

Phytochemical and anticarcinogenic evaluation of triphala powder extract, against melanoma cell line induced skin cancer in rats

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ABSTRACT

Objective: Triphala is a botanical preparation consists of Terminalia chebula, Emblica officinalis, Terminalia bellerica and it exhibits a number of health benefits, including antioxidant activity, lowers cholesterol, inhibits HIV, Reduces tumors in animals, protects and improves liver function and many more. Triphala (Harad, Bahada and Amala) in different ratios exhibits a number of health benefits, including: anti-cancerous, antipyretic, antiulcer, antidiabetic activities. Triphala has historically been used as a digestive aid for constipation. Triphala triggered the cancerous cells to die off and significantly reduced the size of the tumours. This study was carried out to evaluate the effect of triphala powder (hydro alcoholic extract) on melanoma skin cancer in rats.

Methods: The study was carried out on the melanoma cell line (B6F10) induce model.

Results: The powder extract of triphala produced a significant activity in Melanoma cell line-induced skin cancer.

Conclusions: Triphala extract increased healing of melanoma skin cancer and prevented the development of experimentally induced skin cancer in rats.

Keywords: Triphala, Melanoma, B6F10, Skin cancer, Hydro alcoholic

Introduction

Cancer is a disease characterized by uncontrolled proliferation of cells that have transformed from the normal cells of the body. The cancer cells can invade the adjacent and distant tissues via the circulation. Cancer is a group of diseases in which cells are aggressive (grow and divide without respect to normal limits), invasive (invade and destroy adjacent tissues), and/or metastatic (spread to other locations in the body). These three malignant properties of cancers differentiate them from benign tumors, which are self-limited in their growth and do not invade or metastasize (although some benign tumor types are capable of becoming malignant). Triphala is a botanical preparation comprised of equal parts of three herbal fruits: Harada (Terminalia chebula, black...
myrobalan), amla (*Emblica officinalis* or Indian gooseberry), and bihara (*Terminalia bellerica*). Researchers found that *T. chebula* increased gastric emptying by 86 percent, compared to 76 percent for metoclopramide. Since *Terminalia* is free of side effects, the herb may be a useful alternative to the prokinetic drugs currently available.¹ Recently published studies report that *Terminalia* exhibits antibacterial activity against a number of bacterial species. One group of researchers found that *Terminalia* is effective in inhibiting the urease activity of *Helicobacter pylori*, a ubiquitous bacterium implicated in the development of gastritis, ulcers and stomach cancers² northern research team has shown that extracts of *T. chebula* strongly inhibit the growth and adherence of *Streptococcus* (*S. mutans*), a virulent cavity-inducing organism. *Terminalia* has been found to possess antiviral activity. Researchers have reported that *Terminalia* protects epithelial cells against influenza A virus, supporting the traditional use of *Terminalia* for aiding in recovery from acute respiratory infection.³ *Terminalia* has also demonstrated therapeutic activity against herpes simplex virus (HSV) *in vivo* test animal studies show that when extracts of *Terminalia* were administered following induction of anaphylactic shock, serum histamine levels were reduced, indicating that *Terminalia* may possess a strong anti-anaphylactic action. *Emblica officinalis* fruit (commonly known as amla) is the world’s richest source of natural vitamin C, researchers have attributed many of its traditional benefits to its antioxidant properties. Indian researchers have shown that extracts of amla exhibit antitumor activity. Solid tumors induced by DLA (Dalton’s lymphoma ascites) cells were reduced significantly when mice were fed either amla or an herbal preparation containing 50% amla. In addition to the previously reported effects of amla on normalizing lipid profiles, Indian scientists have reported that flavonoids extracted from amla exert highly potent hypolipidemic and hypoglycemic activities.

This study was to evaluate its effect on the development and healing of melanoma induced skin cancer in rats.

### Materials and Methods

#### Plant material

The Triphala powder from Dabur was brought from a shop, new market, Bhopal, Madhya Pardesh, India.

#### Experimental animals

Mice (swiss albino) were obtained from Dept. of Research, JN Cancer Hospital & Research Center, Bhopal (M.P.). All the mice were kept at controlled light and humidity condition (light: dark, 12:12 hr) and temperature 22±1°C (according to CPCSEA norms). The experimental protocol was approved by the institutional Animal Ethics committee 500/01/a/CPCSEA/2001 Following C.P.C.S.E.A guidelines.

#### Extraction of plant material and preparation of the test dose

Powdered material weighed and soaked with solvent (50% Methanol) in a pear shaped separating funnel. Solvent added to soaked powder up to the level so that a separate layer of solvent only appears above the powder bed. Mixture was agitated at regular interval for 72 hrs. The filtrate was taken out and fresh solvent was again added to the treated powder. Filtrate was concentrated in water bath at 50°C. Concentrated extract was dried at 50°C in oven. Dried extract was powdered and packed in an air tight container. Mixed 1 gm powder extract with double distilled water and made up to 1 ml. This is 100% stock solution stored in a vial at 2-8°C.

#### Phytochemical screening

To identify the chemical constituents the powdered extract was treated with various phytochemical tests i.e. including test for alkaloids, carbohydrates, tannins and phenolics compounds, saponins etc. The results were indicated in Table 1.
Acute toxicity studies

The adult Swiss albino mice 20-25 g were selected for the studies. In the acute toxicity test, we used seven doses and 10 mice in methaonolic extract i.e., 50, 150, 250, 500, 1000, 1500, 2000 mg/kg body weight. During the course of study the behaviours of the mice were carefully observed and fall of time, reduction of spontaneous activity also determined using instruments like Rota rod, actophotometer. All groups of test drug showed neither any toxic effect nor any lethal effect in the dose range of 50 to 250 mg/kg body weight. In 500 mg/kg to 2000 mg/kg of the methaonolic extract group altered behaviour and mortality of mice had been observed. So we had taken a 50 mg/kg of methaonolic extract for further screenings.

Melanoma skin bio assay

Melanoma cell line were obtained from National Cell science centre, Pune and maintained in our laboratory. The C57 Bl hybrid mice of both sexes of the mean weight of 25 gm and 6-7 weeks old were obtained from the animal colony of our institute. Melanoma cell line were obtained from National Cell science centre, Pune and maintained in our laboratory. The following groups were maintained.

Control group: This group consisted of six mice. The melanoma cell line (B6F10) were injected subcutaneously in all six mice.

Test group: This group consisted of six animals. The melanoma cell line was injected by subcutaneous route. The tumour bearing mice were orally given dose of 50 mg/kg body weight in methaonolic extract of triphala. Survival time was also increased in triphala treated mice as compare with untreated tumour bearing mice.

Statistical analysis

Results of all the estimations done were indicated in terms of mean ± SEM. Statistical significance of data were evaluated by analysis of variance (one way ANOVA), followed by comparison between different groups using Krusskal Wallis test. P <0.05 level of significance was considered. The untreated group was compared with the treated group.

Results and Discussion

That preliminary phytochemical screening of triphala showed the presence of different chemical active constituent, such as alkaloids, glycosides-cardiac and anthraquinone, tannin, vitamin C, saponin, carbohydrates (Table 1). On acute oral toxicity test in mice (Table 2) at dose levels 50, 150, 250 mg/kg minimal mortality was observed, when the dose increased the mortality was higher at 500, 1000, 1500, 2000 mg/kg toxic symptoms like depression, loss of muscle tone, drowsiness was observed. Due to this the dose 50 mg/kg for methaonolic extract for further study.

Table 1: Preliminary phytochemical screening of triphala.

<table>
<thead>
<tr>
<th>Chemical Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycoside: Anthraquinone glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac Glycoside</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>+</td>
</tr>
<tr>
<td>Vitamin - C</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2: Oral acute toxicity study of extracts of the methaonolic extract of triphala.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/ml)</th>
<th>Total death in methaonolic extract</th>
<th>% dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>50</td>
<td>0/10</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>150</td>
<td>1/10</td>
<td>10</td>
</tr>
<tr>
<td>III</td>
<td>250</td>
<td>3/10</td>
<td>30</td>
</tr>
<tr>
<td>IV</td>
<td>500</td>
<td>5/10*</td>
<td>50</td>
</tr>
<tr>
<td>V</td>
<td>1000</td>
<td>8/10</td>
<td>80</td>
</tr>
<tr>
<td>VI</td>
<td>1500</td>
<td>10/10</td>
<td>100</td>
</tr>
<tr>
<td>VII</td>
<td>2000</td>
<td>10/10</td>
<td>100</td>
</tr>
</tbody>
</table>

n=no. of animals (ten in each group), *indicates LD_{50}

This study investigated the effect of triphala on the melanoma cell line induced skin cancer in rats. The study showed the effect of triphala
The preventive effect of triphala extract was calculated using the parameter of inhibition rate (IR), increase in the life span (ILS), and Volume of tumour. The inhibition rate was 69% in triphala extract treated group as compared to control group. The life span was also increased in 52% as compared to control group. The volume of triphala treated mice was significantly reduced as compared to control.

The differences in the values of the results of experimental groups were statistically analyzed and found significant in comparison to the control groups ($p <0.05$). The presence of elemental contents is suggests that triphala as a whole is rich in Mg, K, Ca, Fe, and Zn, though Se (selenium) is also present in significant amounts. There appears to be an association between Se deficiency and protein malnutrition disease, multiple sclerosis, cancer and heart disease. It has been suggested that Se (selenium) as glutathione peroxidase inhibits the replication of tumour viruses and prevents the malignant transformation of cells. Triphala is novel dietary or natural chemo preventive formulation. Triphala has been used for centuries in Ayurvedic medicine to treat various types of gastrointestinal-related disorders; however, the molecular mechanisms of triphala have not been studied yet. In the present study, it has demonstrated that methanolic extract of triphala is effective in inhibiting the growth of melanoma cancer cells in the in vivo model. The results reveal that triphala treatment drastically reduces the tumour volume as well as increases the life span of the animals. To the best of our knowledge, this is the first study to report the molecular mechanism of the chemotherapeutic effects of triphala against melanoma skin cancer. The reduction in tumour count may be due to effect in the promotional phase of tumourgenesis which prevent the reduction of free radicals. Triphala becomes one of the highly potential herbal medicines in cancer treatment and prevention because all three compositions of Triphala have been found to possess notable anticancer properties. Although very little is known about the mechanism by which these plants act against cancer cells. The mechanism of in vitro cytotoxicity and tumor growth reduction in vivo induced by triphala seems to involve apoptosis induction. In addition, the components of triphala may exert synergistic cytotoxic action on tumor reduction. Gallic acid is one of the major components of triphala and capable of inhibiting cancer cell proliferation suggesting the key factor responsible for antimutagenic and cytotoxic effects of triphala. Polyphenols such as tannins and gallic acid, a component unit of hydrolysable tannins, are well known inducers of apoptosis in tumour cells. The anticarcinogenic effect of triphala extract suggests its role in chemoprevention of skin cancer. These results are important because this drug is used globally as a bowl cleaning agent. It may also be an important drug for chemotherapeutic treatment of cancer.

**Conclusions**

The rapid increase in utilization of herbal remedies worldwide has been inspired by several factors, including the concept that herbal products are safe and effective and so investigation on medicinal plants is increasing.
day by day. Triphala is known as the mother of medicine as it has a biodiversity of both nutritional as well as medicinal components. It is suggested that any herb or plant ingredients taken must be tested before being used as a remedy. Therefore, from this study, it is clear that the medicinal plants play a vital role against various diseases. Regarding the results obtained in our study we conclude that the methanolic extracts of the powdered triphala is able to prevent melanoma cell line induced skin cancer in rats. However, further studies are required to establish its exact mode of action and the active principles involved in its anticarcinogenic effect.

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References