AML A – THE ROLE OF AYURVEDIC THERAPEUTIC HERB IN CANCER

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ABSTRACT
Medicinal plants are part of human society to combat diseases, from the dawn of civilization. Phyllanthus emblica (Amla) possesses a vast ethnomedical history and represents a phytochemical reservoir of heuristic medicinal value. It is one of the oldest oriental medicines mentioned in Ayurveda as potential remedy for various ailments. The fruit is rich in quercetin, phyllembelic compounds, gallic acid, tannins, flavonoids, pectin, and vitamin C and also contains various polyphenolic compounds. A wide range of phytochemical components including terpenoids, alkaloids, flavonoids, and tannins have been shown to possess useful biological activities. Many pharmacological studies have demonstrated the ability of the fruit shows antioxidant, anticarcinogenic, antitumor, antigenotoxic, antimutagenic, and induction of apoptosis properties. Therefore, scientific validation of such medicinal plants is needed in order to find out their possible use in cancer prevention.

KEYWORDS: Phyllanthus emblica, Medicinal herb, anticarcinogenic, antitumor activity, chemoprevention.

INTRODUCTION
Cancer is a multifactorial, multifaceted and multimechanistic disease requiring a multidimensional approach for its treatment, control and prevention. Cancer remains a major public health burden in the United States and in other developed as well as developing countries[1]. World Health Organization (WHO) reported that there are now more than 11 million are diagnosed with cancer every year and it is estimated that there will be 16 million new cases per year by 2020[2]. Epidemiological studies indicate that exposure of chemical carcinogen such as tobacco smoke, diet, workplace is a major cause of human cancer[3,4]. Chemoprevention by synthetic agents can produce toxic side effects, which have limited their extensive use[5,6,7,8]. Chemoprevention also deals with the chemotherapy of pre-cancerous lesions, which are called pre-invasive neoplasia, dysplasia or intra-epithelial neoplasia depending on the organ system[9]. Chemoprevention is a rapidly growing area of oncology which is regarded due to its magnificent vitamin C content[10]. Traditionally, Amla is regarded as "one of the oldest oriental medicines mentioned in Ayurveda as potential remedy for various ailments. The fruit is rich in quercetin, phyllembelic compounds, gallic acid, tannins, flavonoids, pectin, and vitamin C and also contains various polyphenolic compounds. A wide range of phytochemical components including terpenoids, alkaloids, flavonoids, and tannins have been shown to possess useful biological activities. Many pharmacological studies have demonstrated the ability of the fruit shows antioxidant, anticarcinogenic, antitumor, antigenotoxic, antimutagenic, and induction of apoptosis properties. Therefore, scientific validation of such medicinal plants is needed in order to find out their possible use in cancer prevention.

Phyllanthus emblica (Family: Euphorbiaceae, Syn: Emblica officinalis), commonly known as amla, is one of the foremost plants utilized from antiquity till to date. Amla is regarded as "one of the best rejuvenating herbs” in the Ayurveda, an Indian traditional medicinal system. It is distributed in tropical and subtropical areas of China, Indonesia and the Malay Peninsula. It is highly regarded due to its magnificent vitamin C content[11]. Traditionally, the fruit is useful as an astringent, cardiac tonic, diuretic, laxative, liver tonic, diuretic, refrigerant, stomachic, restorative, antipyretic, anti-inflammatory, hair tonic and digestive medicine[12,13]. The fruits of amla contain a wide variety of phenolic compounds, such as tannins, phyllembelic acid, phyllembolin, rutin, curcuminoids and emblicol[14]. The fruit is reputed to have a broad range of therapeutic effects including anticarcinogenesis and antimutagenic[15], antitumour[16] and induction of apoptosis[17].

Triphala is a traditional Ayurvedic herbal formulation, consisting of equal parts of three medicinal plants namely Emblica officinalis, Terminalia chebula and Terminalia belerica. Triphala strengthens the different tissues of the body, prevents ageing, promote health and Immunity[21]. The goals of this review is to provide a critical insight on the cancer preventive potential of Phyllanthus emblica, covering its phytochemistry, traditional medicinal uses, experimental and laboratory investigations and toxicological properties. Amla is a medium to large deciduous tree. Leaves are simple, linear-oblong blunt, small, 8-10mm or more long. Flowers are greenish yellow, in axillary fascicles, 0.5-1.5cm long, fruits are nearly spherical or globular, about 18-25mm wide and 15-20mm long[22]. The bark is shining greyish brown or greyish green. Amla is a medium to large deciduous tree. Leaves are simple, linear-oblong blunt, small, 8-10mm or more long. Flowers are greenish yellow, in axillary fascicles, 0.5-1.5cm long, fruits are nearly spherical or globular, about 18-25mm wide and 15-20mm long[22]. The bark is shining greyish brown or greyish green. Amla is a medium to large deciduous tree. Leaves are simple, linear-oblong blunt, small, 8-10mm or more long. Flowers are greenish yellow, in axillary fascicles, 0.5-1.5cm long, fruits are nearly spherical or globular, about 18-25mm wide and 15-20mm long[22]. The bark is shining greyish brown or greyish green. Amla is a medium to large deciduous tree. Leaves are simple, linear-oblong blunt, small, 8-10mm or more long. Flowers are greenish yellow, in axillary fascicles, 0.5-1.5cm long, fruits are nearly spherical or globular, about 18-25mm wide and 15-20mm long[22]. The bark is shining greyish brown or greyish green.
life, health and young appearance. The fruits are used as an expectorant, an antitoxin to "mineral" poisons, particularly vermillion and sulfur. The crushed fruits have a good effect on hair growth and prevent hair graying[31]. In Tibetan medicine, the fruit have been described as having a sour taste with cooling potency. It is used as a febrifuge, as an anti-inflammatory and unusually as an anti-diuretic[32]. Indonesians used the fruits for the treatment of dysentery, diarrhea, cholera and biliary disorders[33]. The pulp of the fruit is smeared on the head to dispel headache and dizziness caused by excessive heat[34].

**PHYTOCHEMICAL CONSTITUENTS**

The phytochemicals of this plant include hydrolysable tannins (Emblicin A, Emblicin B, punigluconin, pedunculagin)[35], flavonoids (Kaempferol 3 alpha L (6'-methyl) rhamnopyranoside, Kaempferol 3 O alpha L (6'-ethyl) rhamnopyranoside)[36], alkaloids (Phyllanthidine and phyllantine)[37], Gallic acid, ellagic acid, 1-O-galloyl-beta-D-glucose, 3,6-di-O-galloyl-D-glucose, chebulagic acid, quercetin, chebulagic acid, corilagin together with isoelectricin, were isolated from the fruit of Phyllanthus emblica[38].

A new acylated glucoside was isolated from the methanolic extract of the leaves of Pemblcia. Their structures were named as apiogenin 7-O-(6'-butyl)glucopyranoside and 7-O-(6'-butyl-beta)-glucopyranoside, along with four known compounds gallic acid, methyl gallate, 1,2,3,4,6-penta-O-galloylglucose and luteolin-4'Oneohesperiodoside[39]. The seeds of P.emblica contain fixed oil, phosphatides and a small quantity of essential oil. In addition, the leaves contain gallic acid, ellagic acid, chebulagic acid and chebulinic acid. Phyllanthus emblica, a novel highly oxygenated norbisabolane were isolated from the roots of Pemblcia and its structure was fully characterized by spectroscopic and chemical means[39]. Ellagic acid and lupecol are present in roots of Pembla[40,41]. Structures of chemical constituents are found in figure 1.

**NUTRITIONAL VALUE**

Amla is well known for its nutritional qualities. It is rich in polyphenols, minerals and is regarded as one of the richest source of vitamin C (200-900 mg per 100 g of edible portion)[42,43]. Major components of nutritional importance are reported in table 1.

**CANCER PREVENTIVE EFFECTS**

Phyllanthus emblica has been prescribed in the traditional ayurvedic medicine for the treatment of various ailments. To the best of our knowledge, there is some little scientific investigation that deals with the chemopreventive activity of Phyllanthus emblica in laboratory and experimental studies. A summary of the findings of these studies is presented below.

**IN VITRO**

**ANTICANCER/ANTITUMOUR EFFECTS**

The potential anticancer effects of aqueous fruit extract of P. emblica was tested in several different human cancer cell lines such as A549 (lung), HepG2 (liver), HeLa (cervical), MDA-MB-231 (breast), SK-OV3 (ovarian) and SW620 (Colorectal). Pemblcia extract significantly inhibited the growth of several human cancer cell lines at doses of 50-100µg/ml. P. emblica extract inhibited invasion of MDA-MB-232 cells in vitro matanti gel invasion assay at doses of 25 and 50 µg/ml[44]. Aqueous extracts of Pemblcia can inhibit 1292 cells growth in a dose dependent manner. Its IC50 value was 16.5 µg/ml[45] and it was most active in inhibiting in vitro cell proliferation[46].

Apoptosis (programmed cell death), is a useful marker for predicting tumour response after anticancer treatment. The increased activity of caspase-3, caspase-7 and caspase-8 but not caspase-9, up-regulation of FAS protein via a death receptor mechanism and apoptotic DNA fragmentation in HeLa cells treated with Pemblcia extract[47].

The efficacy of Emblica officinalis Polyphenols (EOP) on the induction of apoptosis in mouse and human carcinoma cell lines. EOP was reported to induce apoptosis in DLA and CeHa cell lines. In addition, it also inhibited DNA topoisomerase I in Saccharomyces cerevisiae, mutant cell cultures and the activity of cdc-25 tyrosine phosphatase[48]. Pemblcia extract was found to inhibit cell cycle regulating enzymes cdc 25 phosphatase in a does dependent manner. The IC50 dose of extract was found to be 5 µg/ml[49]. In vitro biological activities are reported in table 2.

The modulatory effect of EOP fractions was evaluated on liver tumours induced by Nitrosodiethylamine (NDEA) in rats 5 days a week for 20 weeks followed by NDEA administration. Alkaline phosphatase (ALP), glutamate pyruvate transaminase (GPT), liver glutathione S-transferase (GST) was evaluated in the treated animals. The level of above mentioned enzymes was increased, whereas the treatment of EOP reduced the activities of all enzymes[50].

**IN VIVO**

**LIVER CANCER**

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and also existing as a leading cause of death[46]. The major occurrence of HCC in Africa and Asia are linked to chronic infection with hepatitis B (HBV) and/or hepatitis C (HCV) virus and perhaps to aflatoxins exposure[47,48]. More and more efforts have been made in search of natural materials and foods as a means of chemical prevention of liver cancer[49].

Only a few studies have speculated the chemopreventive effects of Pemblcia against liver cancer. It was tested in vivo in wistar rats treated with carcinogen Diethylnitrosamine (DEN) (200mg/kg b.wt. ip) to induce liver cancer. The results showed that pretreatment of methanolic fruit extract (100 and 200 mg/kg b.w) exhibited significant pathological manifestations at both the doses. Emblica officinalis (EO) has the potential to be useful in ameliorating the carcinogen-induced response in rat[50].

**SKIN CANCER**

Skin cancer is the most common type of cancer in the United States[51], with more than a million reported cases[52] and 9,000 deaths per year[53]. According to the World Cancer Report, it constitutes ~30% of all newly diagnosed cancers in the world, and solar ultraviolet (UV) radiation (particularly, its UVB component; 290-320 nm) is an established cause of ~90% of skin cancers[54].

The cancer preventive effect of EO was investigated on two stage process of skin cancer induced by 7, 12-dimethylbenz (a) anthracene (DMBA) in Swiss albino mice. It showed significant chemopreventive effects on DMBA-initiated and croton oil (1% in 100µl of acetone) promoted skin cancer development. P.emblica exhibited a significant reduction in tumour incidence, tumour yield, tumour burden and cumulative number of papillomas. These finding were indicative of chemopreventive potential of P. emblica against skin carcinogenesis[55].

**ANTIGENOTOXICITY**

The protective effect of P. emblica fruit extract against clastogenicity induced by lead nitrate on the incidence of sperm head abnormalities. The results clearly indicate that extract exhibited significant reduction in the frequency of sperm head abnormalities. The finding of the above study shows that P. emblica plays a key role in inhibition of heavy metal mutagenesis in mammals[56].

The ethanolic extract of Emblica officinalis (EO) fruit extract was evaluated for protection against genotoxicity induced by the rodent carcinogen, 7,12-dimethylbenz(a)anthracene (DMBA). The results showed that extract significantly increases glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR) and detoxifying enzyme glutathione-S-transferase (GST). The extract also reduced the hepatic levels of the activating enzymes cytochrome (CYt) P450 and CYt b6.

The protection afforded by EO may be associated with its antioxidant capacity and through its modulatory effect on hepatic activation and detoxifying enzymes[57]. An aqueous extract of
Emblica officinalis fruit protected mice against the chromosome damaging effects of the well known carcinogen 3,4-benzopyrene[59].

Table 1: Nutritional Value of fruit of Phyllanthus emblica (% or per 100g)

<table>
<thead>
<tr>
<th>Chemical components</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Protein</td>
<td>0.5%</td>
</tr>
<tr>
<td>Fat</td>
<td>0.1%</td>
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<tr>
<td>Mineral matter</td>
<td>0.7%</td>
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<tr>
<td>Fibre</td>
<td>3.4%</td>
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<tr>
<td>Carbohydrate</td>
<td>81.2%</td>
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<tr>
<td>Fruits: Moisture</td>
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<tr>
<td>Vitamin C</td>
<td>600mg/100g</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>0.02%</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.05%</td>
</tr>
<tr>
<td>Bulk elements</td>
<td>Mg/100g, net weight</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.05%</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>0.02%</td>
</tr>
<tr>
<td>Iron</td>
<td>1.2 mg/100g</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>600mg/100g</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>0.2mg/100g</td>
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</tbody>
</table>

Table 2: In vitro biological activities of P.emblica

<table>
<thead>
<tr>
<th>IN VITRO CELL LINES</th>
<th>BIOLOGICAL ACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous fruit of P.emblica</td>
<td></td>
</tr>
<tr>
<td>HepG2 (liver)</td>
<td>Inhibition of cell growth in human cancer cell lines</td>
</tr>
<tr>
<td>HeLa (cervical)</td>
<td></td>
</tr>
<tr>
<td>MDA-MB-231 (Breast)</td>
<td></td>
</tr>
<tr>
<td>SK-OV3 (Ovarian)</td>
<td></td>
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<tr>
<td>SW620 (Colorectal)</td>
<td></td>
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<tr>
<td>L929 cells</td>
<td>Inhibition of cell growth</td>
</tr>
<tr>
<td>Emblica officinalis polyphenols</td>
<td>Induction of apoptosis</td>
</tr>
<tr>
<td>Asites (DLA) and GHA cell lines</td>
<td></td>
</tr>
<tr>
<td>MDA-MB-232 cells</td>
<td>Inhibition of invasion</td>
</tr>
<tr>
<td>Pyrogallol (Active compound)</td>
<td>Inhibition of cell proliferation</td>
</tr>
<tr>
<td>Human Erythromyeloid K562, T-Lymphoid Jurkat, B-Lymphoid Raji, Erythroleukemic HEL cell lines</td>
<td></td>
</tr>
</tbody>
</table>

Structure of gallic acid

Structure of ellagic acid

Fig. 1: Structure of chemical constituents found in Phyllanthus emblica:

ANTIMUTAGENECITY

The activation and mutagenicity of 2-Acetamidofluorene (2-AAF) was inhibited by P.emblica. It also inhibits the cytochrome P-450, aniline hydroxylase[21]. The antimutagenic potential of water, acetone and chloroform extracts of Emblica officinialis has been evaluated on sodium azide and 4-nitro-o-phenylenediamine induced his+ revertants in TA100 and TA97 tester strains of Salmonella typhimurium. The study revealed that chloroform extract was less active compared with water and acetone extracts[60].

TRIPHALA

The cancer chemopreventive potential of Triphala was established on benzpyrene induced fore stomach papillogenesis in mice. The results reported that Triphala significantly increased the antioxidant status which might have contributed to the chemoprevention[61]. The in vitro cytotoxic effect of Triphala was tested on Shionogi 115 (S115) and MCF-7 breast cancer cells and PC-3 and DU-145 prostate cancer cell lines.

It was found that acetone extracts showed significant cytotoxic effect on cancer cells, which may be due to the presence of polyphenols gallic acid present in Triphala[62]. Triphala was administered orally to nude mice implanted with Capan-2 xenograft. Further, oral administration of 50 mg/kg or 100 mg/kg Triphala in PBS, 5days/week significantly suppressed the growth of Capan-2 pancreatic tumor-xenograft. Reduced tumor-growth in Triphala fed mice was due to increased apoptosis in the tumors cells, which was associated with increased activation of p53 and ERK[63].

TOXICOLOGY

Phyllanthus emblica has been widely consumed by persons for thousands of years, largely without untoward incident, and thus is considered generally safe. It was found to be non-toxic to human and experimental animals[64]. No toxicity or mutagenicity was observed in the toxicological results of the experimental models in the highest dose range[65]. Toxicological studies in animals stated that LD50 for orally administered extract in rats was reported to be about 1 g/kg body weight[66,67].

CONCLUSION

Phyllanthus emblica L, the versatile medicinal plant deserves a special attention of the scientific fraternity to emerge as a milestone for medical science of this millennium due to its various medicinal uses. In this review, we have presented information on the botanical description, traditional uses, phytochemistry, anticancer effects, and toxicity studies of Phyllanthus emblica.

A variety of phytochemicals such as tannins, flavonoids, terpenoids, alkaloids are reported to indicate several pharmacological properties such as antioxidant, anticancer, antitumor, antigenotoxic, and anticarcinogenic effects. It is considered to be a safe herbal medicine without any adverse effects. Future research should focus on how genetic variability and daily environmental factors influence the anticancer benefits attributed to emblica, which can be used for the welfare of the mankind.

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