International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2018): 7.426

Evaluation of Anticancer Activity of *Delonix regia* Extract against Chemical Induced Skin Carcinogenesis in Mammals

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Abstract: ***Cancer has emerged as a life threatening non-communicable disease and it is characterized by the abnormal proliferation, invasiveness and metastasis of cells. During the last few decades, there has been a sudden increase in the cancer global burden due to the change in life style, environment, genetic variation, virus infection etc. which directly or indirectly responsible for the cancer development [1]. Among all the cancers, skin carcinogenesis are the most prevalent as it represents almost one third of all the newly diagnosed cases. Melanoma and non-melanoma are the two major categories of skin cancer. Anticancer activity of Delonix regia against skin cancer is studied in the present paper.***

Keywords: Delonix regia, skin cancer

1. Introduction

Cancer has emerged as a life threatening non-communicable disease and it is characterized by the abnormal proliferation, invasiveness and metastasis of cells. During the last few decades, there has been a sudden increase in the cancer global burden due to the change in life style, environment, genetic variation, virus infection etc. which directly or indirectly responsible for the cancer development [1]. Among all the cancers, skin carcinogenesis are the most prevalent as it represents almost one third of all the newly diagnosed cases. Melanoma and non-melanoma are the two major categories of skin cancer. The two most frequent type of non-melanoma skin cancer comprises Basal Cell Carcinoma (BCC) and Squamous Cell Carcinoma (SCC), which has annual incidences of about 80 and 16% respectively [2].

The two stage skin carcinogenesis model in mouse is a novel tool to study the stages and mechanism of cancer as it displays a preneoplastic condition in the form of papillomas that are visible and can be confirmed histopathologically. It involves classical tumor initiator 7, 12-Dimethylbenz (a) anthracene (DMBA) which causes the mutations leading to DNA damage.

TPA, the phorbol ester present in Croton oil, acts as the promoter and alters gene expression resulted in hyper proliferation, tissue remodelling and inflammation [3]. Besides the traditional treatment methods like chemotherapy and radiotherapy, there should be an alternative preventive approach to reduce the cancer burden.

The administration of herbal and natural products to reverse, inhibit and delay the carcinogenesis is a new area of research known as chemoprevention [4]. It postpones the cancer occurrence in high risk population and simultaneously reduces the side effects of various treatment therapies.

Chemopreventive agents modulate different stages of cancer development either by blocking mutagenic carcinogens, scavenging of free radicals or reduction in apoptosis and abnormal cell proliferation [5]. As reported in scientific study cancer cases are directly associated with the nutritional value of diet consumed [6].

Chemoprevention is a pharmacological way of interference in order to arrest or reverse the process of carcinogenesis. Chemopreventive substances are identified on the basis of their antioxidant, antimutagenic, and anti-inflammatory activities capable of arresting proliferation and enhancing apoptosis which are the major criteria for their anticarcinogenic activity.

Progress in the area of chemoprevention during the past two decades has been very impressive. Accumulating epidemiological and experimental evidences have revealed the chemopreventive influence of number of naturally occurring compounds and their role in prevention of the diseases [13–17].

Herbal products are gaining progressively attention these days for primary health care owing to less toxicity, better compatibility with the body, and high efficacy against free radical mediated diseases. Many studies have suggested that a healthy diet, especially fruits and vegetables that are rich in natural antioxidants, is efficacious to prevent oxidative stress and thus plays a vital role in cancer prevention [18].

The therapeutic use of herbs is an old as human civilization and has evolved along with it. The majority of people on this planet still rely on their complimentary system of medicine with the use of herbal drugs. India is the largest producer of medicinal plants and also called the “Botanical garden of the World”. The medicinal plants, besides having natural therapeutics values against various diseases, also provide high quality of food and raw materials for the livelihood. These plants may promote host resistance against infection by re-stabilizing body equilibrium and conditioning the body tissues. (Gupta and Sharma, 2006). [19]

Emerging concept of combining traditional medicine with advanced drug discovery program is globally acceptable.

Traditional medicine has a long history of serving peoples all over the world. (Decker et al., 2005) (20) . Medicinal plants drug discovery continues to provide new and important leads against various. pharmacological targets including cancer, malaria, cardiovascular diseases and neurological disorders (Sporn et al., 1976; Gerber et al., 2002; Kris et al., 2002; Frederick et al., 2004). (21).

Delonix regia is a species of small attractive tropical trees, it is commonly occurring flowering plant grown as an ornamental tree and given the name Gulmohar. Till now eleven species were discovered in this genus, one occurs in Northeast Africa, nine species were found in endemic to Madagascar and the remaining species occurs from East and Northeast Africa to India. Chemical constituents of different classes such as; flavonoid, terpenoids and its glycosides, phenolics, phytosterol were reported from flowers and leaves of Delonix regia species. A number of published papers report the medicinal properties for Delonix regia .The leaves are reported for its antimicrobial and antioxidant effect.(22,23,24,25, 26.).

Flower of *Delonix regia* has large red-orange in color having five petals, one petal contains also white color streaks and little bit big as compare to other petals, four spreading scarlet or orange-red petals up to 8 cm long having same size and colour, a fifth upright petal called the standard, which is slightly larger and spotted with yellow and white. Sepals 5, thick, green outside and reddish with yellow border within, reflexed when the flowers are open, pointed, finely hairy, about 2.5 cm long. Stamens 5 with 10 red filaments. Pistil has a hairy 1celled ovary about 1.3cm long. Style about 3 cm long.(27s, 28,29).

The flower contain different flavonoids, tannins, alkaloids, saponins, steroids [28, 37] carotenoids(lycopene, phytoene, phtofluene, β-carotene, prolycopene, neolycopene, δ-lycopene nd γ-lycopene)**,** phenolic acid (gallic acid, protocatehuic acid, salicylic acid, trans-cinnamic acid and chlorogenic acid)**,** anthocyanins (cyanidin-3-glucoside and cyanidin-3 gentiobioside and β-sitosterol.(30, 31, 32, 33).

Body has itself antioxidant system, which reacts with reactive species and neutralizes them. Thisnatural antioxidant system includes enzymes like catalase, superoxide dismutase and glutathione, which protect the body from free radical species and prevent oxidative stress [7].

Synthetic antioxidant like butylated hydroxyl toluene and butylated hydroxyl anisole are carcinogenic in nature. So, there arises a need for natural antioxidant [8].

Flavanoids have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis (Jahan et al., 2010). It is important to note that though flavonoids are potent in inhibiting tumor and/or cancer development and promotion, they are also non-toxic to the normal cells. What really sets apart their differential effects in abnormal cancer cells versus normal cells is their ability to induce apoptotic pathways to impede cancer in abnormal cancer cells and at the same time manipulate levels of metabolizing enzymes and induce detoxifying enzymes rendering them non-toxic to normal cells (Kwon et al., 2007).

Natural products have played a highly significant role over the years in the discovery of new drugs. This is particularly evident in the treatment of cancers and infectious diseases in which more than 60 and 75 % of drugs, respectively, are of natural origin (Newman et al., 2003). As an important example, the extract of *Delonix regia* was recently analyzed for its antimicrobial activity as well as general toxicity (Jahan et al., 2010). As few studies investigated the hepatoprotective and cytotoxic activities of D. regia methanol extract (El-Sayed et al., 2011) and (Ahmed et al., 2011), further studies are needed in order to fully understand the mechanisms underlying these activities.

The present study has been focus to evaluate the anticancer and antioxidative potential of *Delonix regia* extract against chemical induced skin carcinogenesis in mammals.

2. Material & Methods

The animal care and handling was approved by ethical committee of our institution and was done according to guidelines set by the World Health Organization, Geneva, Switzerland, and the Indian National Science Academy, New Delhi, India. The inhibition of tumor incidence by Syzygium cumini seed extract was evaluated on two-stage skin carcinogenesis, induced by a single application of DMBA (initiator), and two weeks later, promoted by repeated application of croton oil (promoter) thrice per week, following the protocol for 16 weeks [13].

Animals

The study will be conducted on random-breed male Swiss albino mice of 7-8 weeks old with 24 ± 2 g body weight. These animals will be housed in polypropylene cages in the animal house under controlled conditions of temperature (25°C ± 2°C) and light (14 light:10 dark).

The animals will be fed a standard mouse feed procured from Aashirwad Industries, Chandigarh (India), and water ad libitum. Eight animals will be housed in one polypropylene plastic cage containing saw dust (procured locally) as bedding material. For precaution against infections, tetracycline hydrochloride water was given to these animals once each fortnight. Chemicals - 7, 12-Dimethyl Benz (a) anthracene (DMBA) and croton oil was procured from Sigma Chemical Co., USA. DMBA will be dissolved at a concentration of 100 µg/ 100µl in acetone. Croton oil was mixed in acetone to give a solution of 1% dilution.

Plant material & Extract Preparation

The flower of *Delonix regia* will be collected locally after proper identification. The identification of the plant *Delonix regia* will be done by a botanist from Herbarium, Department f Botany, University of Rajasthan, Jaipur, Rajasthan (India). The flowers will be shade dried, and powdered in a mixture, and the hydro-alcoholic extract will be prepared by refluxing with the double distilled water (DDW) and alcohol (3:1) for 36 (12 x 3) hrs at 40ºC.

The extract will be cooled and concentrated by evaporating its liquid contents. The prepared *Delonix regia extract* (DRE) will be stored at low temperature until its further use and it will be redissolved in DDW prior for the oral administration in mice.

3. Experimental Design

Group I: Vehicle treated Control/Normal (n=10)
Animals of this group will receive topical application of acetone (100µl/ mouse) on the shaven dorsal skin, normal diet and double distilled water (equivalent to drug i.e.100µl/ mouse by oral gavage) for 16 weeks.

Group II: DRE treated Control/Drug alone (n=10)
Animals of this group will be administered *Delonix regia* extract orally once in a day for 16 weeks study period. These animals further divided into following three subgroups according to the dose of DRE administration.

Group III: Carcinogen treated Control (n=10)
Mice of this group will be applied topically a single dose of DMBA (100µg/100µl of acetone) as initiator over the shaven area (3 x 3 cm2) of the skin. Two weeks later, croton oil (1% v/v in acetone) will be applied over the same area as promoter, three times per week until the end of experiment. This group will receive double distilled water equivalent to DRE (100µl/mouse/day) by oral gavage for 16 weeks.

Group IV: DRE treated Experimental -1 (n=10)
These animals will receive the same treatment as in Group III but also DRE orally, once in a day starting from 7 days before and 7 days after DMBA application, and served as peri-initiation group.

Group V: DRE treated Experimental -2 (n=10)
Animals in this group will receive the same treatment of DMBA / croton oil as for Group III, and will be administered DRE by oral gavage, starting from the time of croton oil treatment till the end of experiment (i.e. 16 weeks), and served as post-initiation group.

Group – VI: DRE treated Experimental - 3 (n=10)
Mice of this group will be treated similarly as for Group III. Moreover, these animals will receive the drug (i.e. DRE) by oral gavage starting from 7 days before of DMBA application and will be continued until the end of experiment (i.e. 16 weeks) to serve as peri- & post-initiation group.

Biochemical Study

Biochemical alterations will be measured in animals of all the above groups at the time of the termination of the experiment. The dorsal skin affected by tumors and liver will be quickly excised and washed thoroughly with chilled saline (pH 7.4), then weighed and blotted dry. A 10% tissue homogenate will be prepared from the part of the sample (skin & liver) in 0.15 M Tris- KCl (pH 7.4), and centrifuged at 2000 rpm for 10 minutes. The supernatant thus obtained will be taken for estimation of lipid peroxidation (LPO) and reduced glutathione (GSH). The following biochemical parameters will be estimated in the liver and skin of mice.

Lipid Peroxidation (LPO)
The level of LPO will be estimated spectrophotometrecally by thiobarbituric acid reactive substances (TBARS) method as described by Ohkhawa et al. (1979) [14]. Briefly, thiobarbituric acid (0.6%), sodium dodecyl sulphate (0.1%), and trichloroacetic acid (20%) were added to 200 l of the tissue homogenate (10%) prepared as described above. This mixture was heated for 60 minutes, cooled, and extracted with N butanol- pyridine (15:1), the optical density (OD) was ecorded at 532 nm and the contents were expressed as nmol/mg of tissue.

Reduced Glutathione (GSH)
The level of GSH will be estimated as total nonprotein sulphahydryl group by the method of Moron et al. (1979) [15]. The homogenate will immediately precipitated with 100 µl of 25% trichloroacetic acid (TCA) and the precipitate will be removed after centrifugation. Free endogenous-SH will be assayed in a total volume of 3 ml by the addition of 200 µl of 0.6 mM 5, 5′ dithio-bis (2- nitrobenzoic acid) dissolved in 0.2 M phosphate buffer (pH 8.0) to 100 µL of the supernatant and the absorbance will be recorded at 412 nm using a UV-VIS Systronics spectrophotometer. Reduced GSH will be used as a standard and the levels of GSH will expressed as µmol/gm of tissue.

Catalase
The catalase activity will be assayed by the method of Aebi [16]. The change in absorbance will be followed spectrophotometrically at 240 nm after the addition of H2O2 (30 mM) to 100 µL of the supernatant (10% of skin homogenate prepared in 50 mM phosphate buffer and centrifuged for 10 min.) in 50 mM phosphate buffer (pH 7).The activity of the enzyme is expressed as U/mg of tissue, where U is µmol of H2O2 disappearance/min.

Total Proteins
Total Proteins will be estimated by the method of Lowery et al., [17] using bovine serum albumin as a standard and the level will be expressed as mg/ gm.

Superoxide dismutase
SOD will be determined by the method of Marklund and Marklund [18] by quantification of pyrogallol auto oxidation inhibition and the results are expressed as units/mg protein. Auto oxidation of pyrogallol in Tris-HCL buffer (50 mM, pH-7.5) was measured by increase in absorbance at 420 nm.

Vitamin-C
For this, tissue, the fresh organs will be weighed, homogenized in acetate buffer (20 mg/ ml) extracted with cold 4 per cent trichloroacetic acid, centrifuged, and filtered. Ascorbic acid was determined by the method of Roe and Kuether [19].

Histopathological Study
Tumors and normal skin will be removed from the sacrificed animals and immediately fixed in 10% formalin fixative for 24h. The tissues will then dehydrated in ascending series of alcohol, embedded in paraffin wax and 5 micron thick sections will cut and viewed under light microscope.

Statistical Analysis

Data from different experimental groups will be analyzed and expressed as mean + SD. The significant level of difference between carcinogen treated control and SCE treated experimental groups will statistically analyzed using Student's t-test.

4. Observations and Results

Delonix regia extract application on skin reduces the skin cancer. In D.regia the secondary metabolites such as flavonoids sterds tannins and phenol Phenol compounds were found. Carotenoids 2 carotene, zeaxanthein also have antioxidant properties.

Gallic acid (Phenolic compound) sorbic acid, sorbic, m coumaric, p coumaric, ferulic, caffeic, 3 hydroxy benzoic, 4 hydroxy cinnamic acid, 4 hydroxybenzoic acid were also present Due to high phenolic compounds antioxidant properties are present.

Flavonoids also display biochemical and pharmacological actions as anticarcinogenic activities.

Flavonoids act as free redical scavengers and terminate the redical chain reaction that occur during oxidation of tryglycerides in food. Tennins are also potent antioxidants.

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