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Evaluation of anti-cancer activity of *Plumbago indica* root extract

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ABSTRACT

The modern era is trending with the use of herbal medicines for the cure of various ailments, herbal medicines prove to have least side effects when compared to the allopathic form. In the present review, the studies has been conducted for the anti cancerous effect of the medicinal plant *Plumbago indica*, a plant proved to contain various medicinal properties. The plant belongs to the Plumbaginaceae family, a family rich in flavonoids, tannins etc. The main content of study is Plumbagin which shows anti cancerous effect and is also the main constituent in this family. The content also reported to have other properties like anti inflammatory, anxiolytics, anti diarrhoeal, anti spasmodic properties, abortifacient and further more. The study is conducted on Syrian Hamsters' cheek pouches by inducing oral carcinogenesis and by analyszing the results before and after the drug treatments.

INTRODUCTION

Plumbago indica originates from India and South-East Asia, where it is widely used as a medicinal plant. It is cultivated as an ornamental throughout the tropics and in temperate regions in greenhouses. In tropical Africa, *Plumbago indica* is cultivated, sometimes as a medicinal plant, in countries with large populations of Indian immigrants: Kenya, Tanzania, Zimbabwe, Mozambique and Madagascar, north eastern Africa, *Plumbago indica* is used medicinally in a similar way by the Indian population as it is traditionally used in India itself, where many households keep some plants in their backyard. Especially the root has many uses: it is acrid, vesicant, alterative, digestive, stimulant and a powerful abortifacient and oral contraceptive. High doses are dangerous and may cause death. An infusion of the roots is taken to treat dyspepsia, colic, cough and bronchitis. A liniment made from bruised root mixed with a little vegetable oil is used as a rubefacient to treat rheumatism and headache. The milky juice of the leaves is applied on the skin in treatment of scabies, ringworm and haemorrhoids.

Plumbago indica is commonly planted as an ornamental garden plant. The root of *Plumbago indica* contains the naphthoquinone plumbagin (2-methyl juglone). Other compounds isolated from the aerial parts include 6-hydroxyplumbagin, plumbaginol (a flavonol), leucodelphinidin and steroids (e.g. β-sitosterol, stigmasterol, campesterol). Plumbagin possesses several pharmacological activities i.e. antimicrobial, anticancer, cardiotonic and antifertility actions. It is also a powerful irritant. In small doses, the compound is a sudorific and it stimulates the central nervous system; large doses may cause

death from respiratory failure and paralysis. Plumbagin has shown anti-implantation and abortifacient activities in rats. Because of its toxicity, the use of plumbagin in traditional medicine is a dangerous practice. In-vitro tests showed that *Plumbago indica* contains one or more antimutagens. At low doses, plumbagin showed significant tumour inhibitory effects against Ehrlich ascites carcinoma in mice. The ethanol extract of the leaves is active against herpes simplex virus type 1 (HSV-1).

GEOLOGICAL DISTRIBUTION

It is cultivated throughout India and found mostly in Eastern Himalayan region and Sikkim. It also grows in South East Asia and Madhya Pradesh. It is seen in Nagaland, Manipur, Assam, Meghalaya, Sikkim, Arunachal Pradesh, Orissa, West Bengal and South India. It grows in other parts of the world such as Africa, Europe, Indonesia, China, Malaysia, Philippines and Arabian Peninsula. It grows wild in India and has been in use by many tribal since thousand years.

Collection

Collection of plant material Plumbago indica was collected from regions of Kerala like Quilon and Alleppy. After collection, suitable herbarium sheet for each plant with some general information were prepared and send to Scientific and Head of Office, Botanical Survey of India, Southern Regional Centre, Coimbatore (BSI/SRC/5/23/2019Tech/14)

Extraction

The collected plants (leaves and stems) was separated from undesirable materials or plants or plant parts and was shed-dried (35- 50°C). The plant was ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until extraction commenced. About 75 gm of powdered plant material of *Plumbago indica* was taken in a clean, flat bottomed amber glass container and soaked in 350 ml of methanol. The container with its contents was sealed and kept for a period of 10 days accompanied by continuous shaking. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton materials. Then they were filtered by using Whatman filter paper number 1 and the solvent was made to evaporate under the room temperature. The residues were stored in a refrigerator until further studies. Anti bacterial activity is maintained in laminar hood maintaining all precautions required to avoid any contamination derives the test. UV light was kept switch on before half an hour working in laminar hood to avoid any accidental contamination. Petridishes and other glass wares sterilized by autoclaving at a temperature of 121°C and a pressure of 15 lbs/sq. inch for 15 min. Blank discs were kept in a covered petridish and subjected to dry heat sterilization at 180°C for 1 h. Then, they were kept in a laminar hood under UV light for 30 min.

Animals

The Golden or Syrian hamster (Mesocricetus auratus) is a rodent belonging to the hamster subfamily, Cricetinae. Their natural geographical range is limited to arid areas of northern Syria and southern Turkey. Their numbers have been declining in the wild due to a loss of habitat from agriculture and deliberate elimination by humans. Thus, wild golden hamsters are now considered vulnerable by the International Union for Conservation of Nature.

However, captive breeding programs are well-established, and captive-bred golden hamsters are often kept as small house pets. But they are larger than many of the dwarf hamsters kept as pocket pets (up to 5x larger), and so weigh about the same as a sugar glider. They are also used as scientific research animals throughout the world.

The size of adult animals ranges from 5 to 7 in (13 to 18 cm) long, with a lifespan of two to three years (3–4 years in domestic homes, 2–3 years in the wild). Body mass is in the range of 120-125 g.

Like most members of the subfamily, the golden hamster has expandable cheek pouches, which extend from its cheeks to its shoulders. In the wild, hamsters are larder hoarders; they use their cheek pouches to transport food to their burrows. Their name in the local Arabic dialect where they were found roughly translates to "mister saddlebags" due to the amount of storage space in their cheek pouches.[6] If food is plentiful, the hamster stores it in large amounts.

Sexually mature female hamsters come into season (estrus) every four days. Golden hamsters and other species in the genus *Mesocricetus* have

the shortest gestation period in any known placental mammal at around 16 days. Gestation has been known to last up to 21 days, but this is rare and almost always includes complications. They can produce large litters of 20 or more young, although the average litter size is between eight and 10 pups. If a mother hamster is inexperienced or feels threatened, she may abandon or eat her pups. A female hamster enters estrus almost immediately after giving birth, and can become pregnant despite already having a litter. This act puts stress on the mother's body and often results in very weak and undernourished young

Hamsters have unique, stretchy cheeks that are used to store food and carry bedding. Syrian (golden) hamsters are often studied in research facilities for the unique characteristics of their cheek pouches. A hamster's cheeks are also a place where specific diseases can develop that owners should be aware of.

Expeimental procedure

The root of the plant *Plumbago indica* is collected and authenticated. The powder from the leaves of *Plumbaco indica* was soaked with methanol and kept for 15 days, after then it was filtered and kept in open air for evaporation. After evaporation the concentrated methanol extract was then stored for further uses. Testing of different chemical groups present in the extract represents the preliminary pharmacognostical studies. The chemical group tests are performed by 10% (w/v) solution of the extract of *Plumbago indica* in methanol. In methanol reducing sugar, alkaloids, flavonoids and gum steroids are found. The phytochemical screening of the plant parts are conducted.

A total of 80 male Syrian golden hamsters (6 weeks old) weighing 60–80 g were purchased from College of Veterinary Sciences, Mannuthy. The animals were housed, four per cage, in a room with controlled temperature and humidity with 12 h light–dark cycles. All animals were given sterilized soy-free diet and tap water

Tumour Induction

The right cheek pouch of noninbred young (6 weeks old) Syrian hamsters was submitted to topical application of 0.5% DMBA in mineral oil three times a week for 14 weeks in keeping with a standard hamster cheek pouch carcinogenesis protocol. The protocol ensures humane practices. The treated pouch was periodically everted under light anesthesia and examined to monitor tumour development. Once the exophytic tumours had developed and reached a diameter of approximately 3–5 mm, the animals were used for biodistribution and pharmacokinetic studies

The hamsters are divided into 5 groups containing 6 animals in each groups

- **Group I –** Received 5% CMC 10 mL/kg of body weight, the group served as normal control
- **Group II –** Received DMBA (0.7 mL/kg) of body weight by oral gavage
- **Group III –** Received standard drug, 5-Fluoro uracil (0.1mg/kg)
- **Group IV –** Received minimum dose (200 mg/kg) of methanolic extract of *Plumbago indica*
- **Group V –** Received maximum dose (400 mg/kg) of methanolic extract of *Plumbago indica*

After 1 week of acclimatization, the animals were randomly divided into five groups. Left pouches of hamsters in Group II,III,IV and Group V was topically treated 0.7 mL/kg DMBA (in mineral oil) with a paintbrush three times/week for 6 weeks whilst each animal from Group I was similarly treated with only mineral oil. Two days after the last DMBA treatment, the hamsters in Group IV received plumbagin suspended in methanolic extract (200mg/kg) daily by gavage, and animals in Groups V received the maximum dose of plumbagin suspended methanolic extract (400mg/kg) for 12 weeks. Group I received no treatment and served as blank control. Group III received a dose of standard drug 5-Fluoro uracil. Beginning from week 7, each animal was examined once a week in order to record the presence, size and date of detection of all tumors. At the end of week 18, the animals were killed and tissue samples were collected for histopathological and immunohistochemical examination.

RESULTS AND DISCUSSIONS

Topical application of 0.7 ml/kg of DMBA to the left pouch of hamsters significantly decreased the body weight by 6.2% at week 6 compared with the non-treated group. This may be caused by the lower diet intake due to DMBA-induced inflammation, but all animals looked healthy. From weeks 8–20, the body weights were not significantly different among the different groups.

After week 20, the body weights in Groups II-V were lower than in Group I, possibly due to tumour development. Body weights were not significantly different among the four DMBA-treated groups during the period of weeks 6–22. However, at week 24, the body weights of Groups IV and V were significantly lower than that of Group III. This body weight lowering effect of plumbagin was also observed in studies with sister plant *Plumbago zeylanica* .The effect of DMBA treatment are observed on microscopical observations (Fig 6.1.1)

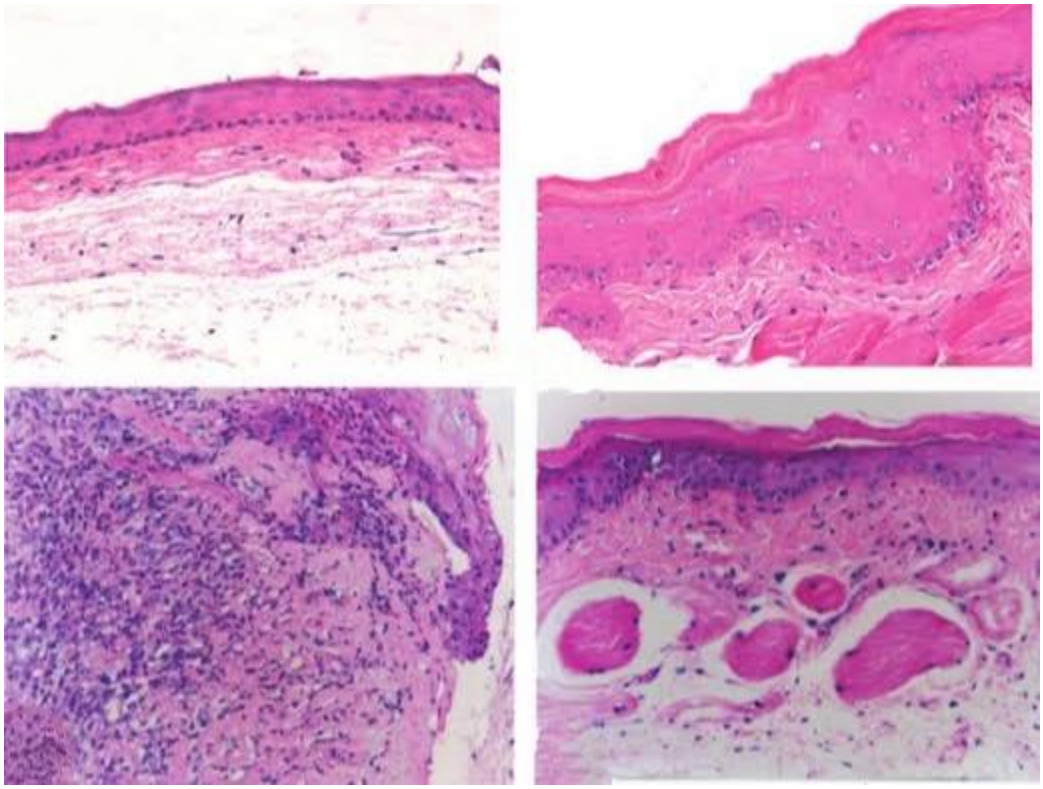

Fig 6.1.1: Oral cancer in Hamsters induced by the treatment of DMBA solution in mineral oil

Inhibition of oral tumor by plumbagin against DMBA-induced oral carcinogenesis

At week 6, all DMBA-treated animals had a visibly roughened granular surface on the mucosa with varying degrees of erythema and occasional white plaque-like lesion. Four of the Six animals analyzed developed dysplasia (75%) and all six animals had hyperplasia (100%). The average numbers of hyperplasia and dysplasia per animal were 3.5 ± 0.7 and 1.5 ± 1.0, respectively. No SCC was observed in these animals.

At week 24, the gavage of plumbagin significantly decreased the visible oral tumour incidence to 69.2% from 92.3% of the positive control (Group II). Although the tumour incidence of Groups IV and V was less than that of Group II, the difference was not statistically significant. When compared with Group II, the average numbers of tumours in Groups III, IV and V were significantly decreased by 52%, 35% and 39%, respectively.

Histologically, the left buccal pouches of DMBA-treated animals presented areas of hyperplasia and dysplasia as well as papillomas . Of the total, ~86% appeared to have developed from papilloma, and the remaining 14% were not associated with papilloma. The combination of plumbagin and plumbagin treatment significantly decreased the oral tumor incidence to 42.3% from 76.9% and plumbagin alone also significantly decreased the SCC incidence to 50.0% .Plumbagin alone (Group IV and V) also decreased the incidence of SCC, but the difference was not statistically significant. When compared with Group II, the average number of SCC per animal in Groups V, IV and III decreased by 53, 51 and 62%, respectively. The average number of papillomas per animal in Group V was also decreased by 48.7%;

however, the incidence of papillomas was not statistically different among the four groups.

In comparison with Group II, the number of dysplasic lesions per animal in Groups IV and V was significantly decreased by 31.5% and 37.5% respectively. However, the number of hyperplasia was not different among the four groups. Later, the observations are made through histopathology studies and the effect of standard drugs and plumbagin are seen in the oral mucosal cells (Fig 6.2.1)

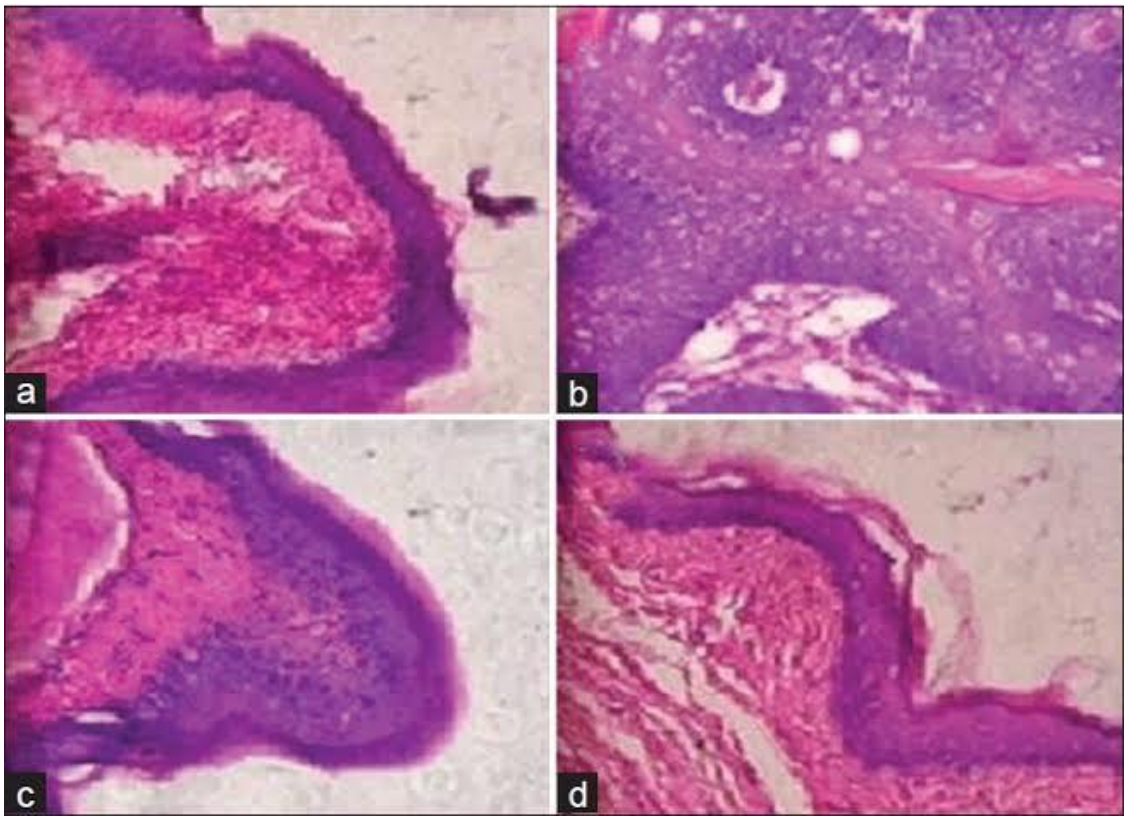

Fig 6.2.1: Effect of standard drug and plumbagin on the oral mucosa of hamsters

Induction of apoptosis and inhibition of cell proliferation by plumbagin

In Group II, the apoptotic index of DMBA-induced oral lesions was significantly higher than that of the non-lesioned area. The apoptotic index in areas with dysplasia or SCC was significantly higher than that of hyperplasia. When compared with Group II, plumbagin (Group III) and the Group IV treatments significantly increased the apoptotic index in dysplasia and SCC; however, the effect of plumbagin (Group IV and V) on apoptosis was not statistically significant in all the lesions. The results and values are confined into a table 6.3.1:

Table 6.3.1: Comparison of tumor numbers and size before and after drug treatment

Groups	No of hamsters	No of tumors	Avg. Dimension of tumors before drug treatment	Avg. Dimension of tumors after drug treatment
I	6	0	-	-
II	6	15	2.14+0.5	2.14+0.05
III	6	13	1.14+0.5	0.6+0.05
IV	6	14	1.86+0.5	1.02+0.05
V	6	15	2.14+0.5	0.8+0.05

In terms of pathological progression, cell proliferation was well correlated with histological severity. The proliferation index increased stepwise from normal epithelium (~5%), basal cell hyperplasia (~9%), dysplasia (~17%), papilloma (~20%) to SCC (~30%). In non-lesioned areas, the proliferative index of Group II was significantly higher than those of Groups IV and V. These data showed that DMBA treatment significantly enhanced cell proliferation even in the non-lesioned area, and it was inhibited by the use of plumbagin minimum and maximum dose. In hyperplasia, dysplasia and papilloma, the proliferative indices of Groups III, IV and V were significantly lower than that of Group II. In SCC, the proliferative index of Group V was significantly lower than that of Group II. All the treatments with plumbagin and plumbagin inhibited cell proliferation significantly decreased hyperplasia, dysplasia and papilloma.

In DMBA-induced oral lesions, the neovascularies primarily concentrated in the stromal areas and spread along stromal ridges on the periphery of epithelial lesions. A few microvessels were observed within the epithelium. The DMBA-induced various oral lesions were significantly higher than that of the non-lesioned area. In Group II, the areas of papilloma or SCC was significantly higher than those of hyperplasia and dysplasia and the SCC was significantly higher than that of papilloma. Compared with Group II, plumbagin (Group IV) and the combination (Group V) significantly decreased papilloma and SCC. However, the effect of plumbagin (Group IV) was not significant in all the lesions.

A graph is plotted between the groups and the dimensions of oral mucosal cancer before drug treatment and after drug treatment (Fig: 6.3.2)

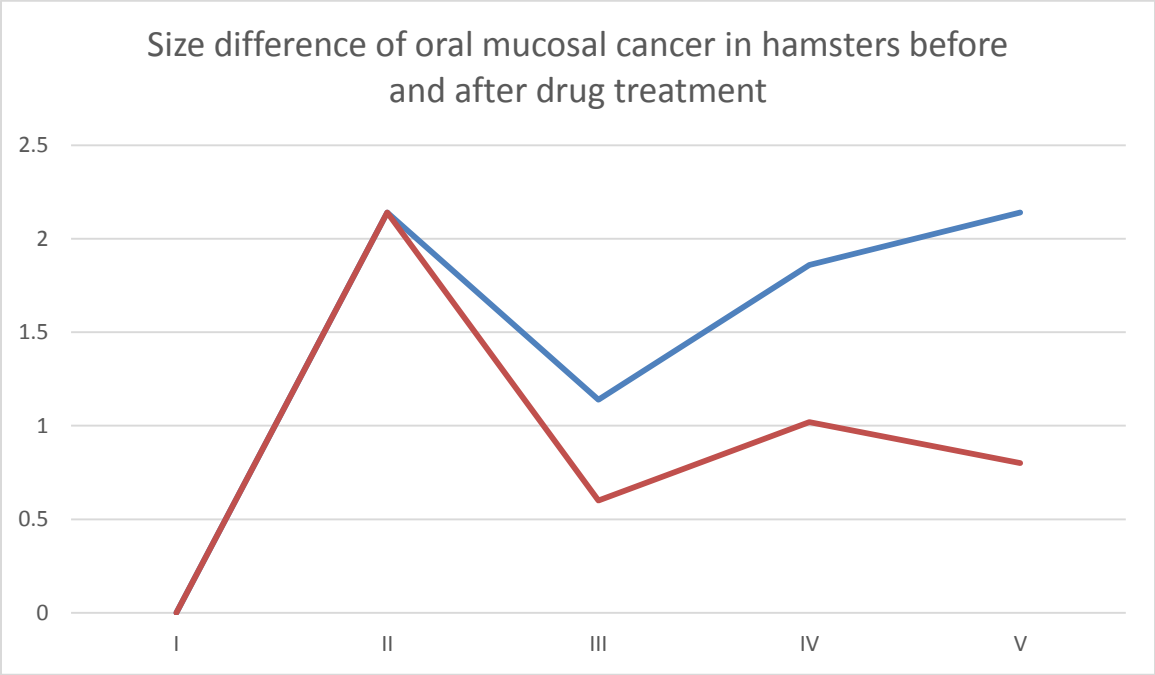

Fig 6.3.2 : Blue line indicates size of oral cancers in different groups before drug treatment and red line indicates size of oral cancers in different groups after the drug treatment

CONCLUSIONS

Plumbagin (5-hydroxy-2-methyl-1,4-napthoquinone) derived from Plumbago species is a potential anti-tumour agent. Plumbagin has been tested for anti-cancer activity *in vitro* using hamster model.

To study the tumour suppressing efficacy of plumbagin using hamster model. The development of tumour was confirmed by performing histology. The tumour was then allowed to progress *in vivo* and the hamsters were administered with plumbagin orally for three continuous days. The tumour suppression capacity was monitored.

The administration of plumbagin had an ability to suppress tumour and the size of the tumour were

relatively lesser when compared with the control sample; it has also increased p53 gene expression.

The study helps to conclude that plumbagin is an effective anti-tumour agent against human cancer cells based on the study *in vitro* in hamsters.

The advantage of inducing tumourigenicity has prompted many reports on induction of tumourigenicity in hamsters.

The use of chemotherapeutic drugs result in a higher range of side effects and have a drastic influence on the quality of life of an individual, therefore a search for an alternative therapy is necessary. Phytochemicals, the non-nutritive components extracted from plants are being studied as one such option. Various photochemical and dietary supplements have been found to exert chemosuppressive effect on cancer. Few phytochemicals like vinblastine and vincristine from *Vinca rosea* are being used in patients and many are on the development pipe line but a comprehensive cure is still evasive.

Our present study of plumbagin using a hamster model, plumbagin stake its claim as a new drug in chemotherapy. But plumbagin too has its side effects as does with the other phytochemicals. Plumbagin has also been reported to have a negative impact on the fertility. The influence on fertility would have a higher influence on AYA (Adolescent and Young Adult) patients.

The suppression of cancer can be correlated with the change in expression of the key cell-regulatory molecules. Termed as Guardian of genome, the p53 is one such regulatory molecule that takes care of cell cycle arrest, apoptosis, differentiation, senescence and DNA repair. It is capable of inducing apoptotic cell death in response to oncogenic stress by activating the transcription of pro-apoptotic genes. Our report suggests that p53 is up regulated on administration of plumbagin, falling in line with similar experiment in mice model. The up regulation of p53 confirms the anti-tumourogenic potential of plumbagin in a genetic level. Neither the present study nor any previous study has strived to unlock the molecule or the receptor to which plumbagin interacts to cause apoptosis by triggering varied cascade. Finding such a molecule will help for the targeted drug delivery of plumbagin and to improve its efficacy.

REFERENCES

[1]. Dhooge,J., De-Vos,M. and Van-Cauwenberge,P.B. Multiple primary malignant tumours in patients with head and neck cancer: results of a prospective study and future perspectives. *Laryngoscope*, 1998, 108250 –256.

[2]. Zarrin Tasnim Rafa *etal* Phytochemical and pharmacological investigation of *Plumbago indica* , 7, *2015*, 125 – 144.

[3]. Slaughter,P., Southwick,H.W. and Smejtal,W. 'Field cancerization' in oral stratified squamous epithelium: clinical implications of multicentric origin.*Cancer (Phila.*), 6, 1953, 963 –968.

[4]. Devi P U *et al* Plumbagin; A plant naphthaquinone with anti tumour and radio modifying properties *Cancer (Phila.*), 70, *1999*, 815 –820.

[5]. Silverman,S. and Griffith,M. Smoking characteristics of patients with oral carcinoma and the risk for second oral primary carcinoma.*J. Am. Dent. Assoc.*85, 1972, 637–640.

[6]. Wong Satit Chuakul *et al.* Plant Resources of South East Asia *Cancer (Phila.*), 73, *1999*, 2674 –2679.

[7]. Fournier,D.B., Erdman,J.W. and Gordon,G.B. Soy, its components and cancer prevention: a review of the *in vitro* , animal and human data.*Cancer Epidemiol. Biomarkers Prev*, 7, 1998, 1055 –1065.

[8]. Adlercreutz,H. Evolution nutrition, intestinal microflora and prevention of cancer: a hypothesis. *Proc. Soc. Exp. Biol. Med*, 217, 1998, 241–246.

[9]. Barnes,S., Peterson,G., Grubbs,C. and Setchell,K. Potential role of dietary naphthoquinones in the prevention of cancer. *Adv. Exp. Med. Biol.*, 354, 1998, 135–147.

[10]. Louise Chang M.D Cancer; The basics.*J. Nutr*, 125, 2018, 777S–783S.

[11]. Connolly,J.M., Liu, X.H. and Rose, D.P. Effects of dietary menhaden oil, soy and a cyclooxygenase inhibitor on human breast cancer cell growth and metastasis in nude mice. *Nutr. Cancer*, 29, 1997, 48–54.

[12]. Fourier D B *et al* Cancer; Prevention and Cure.*Carcinogenesis* 1, *1991*, 469 –472.

[13]. Shamsuddin,A.U. Inositol phosphates have novel anticancer function 1995.

[14]. *J. Nutr* , 123,725S–732S. Michael Friedman Oral Cancer; The basics*J. Cell Biochem* . 22, 2017, 181–187.

[15]. Adlercreutz,C.H.T., Goldin,B.R., Gorbach,S.L. *et al*. Soybeanphytoestrogen intake and cancer risk.*J. Nutr.*, 125, 1995, 757S –770S.

[16]. Alins Bradford Hamsters; diet,habits and behaviour. *Adv. Exp. Med. Biol*, 354, 2014, 135–147.

[17]. McCabe,M.J. and Orrenius,S. Plumbagin induces apoptosis in immature human thymocytes by inhibiting topoisomerase-II.*Biochem. Biophys. Res. Commun*, 194, 1993, 944–950.

[18]. Kramer I B Oral precancerous lesions in cheek pouches of Hamsters*Proc. Natl Acad. Sci. USA*, 90, 1978, 2690–2694.

[19]. Petrakis,N.L., Barnes,S., King, E.B., Lowenstein,J., Wiencke,J., Lee,M.M., Miike,R., Kirk,M. and Coward, L. Stimulatory influence of soy protein isolate on breast secretion in pre- and postmenopausal women.*Cancer Epidemiol. Biomarkers Prev.*, 5, 1996, 785–794.

[20]. Zing Tong Zhoun Effect of genestein in DMBA induced oral cancer in cheek pouches of Syrian hamsters.*Cancer Res.*, 61, 2014, 5045 –5050.