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EVALUATION OF ANTINEOPLASTIC ACTIVITY OF ETHANOLIC LEAVES EXTRACT OF TYLOPHORA INDICA

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ABSTRACT

Aim and objective: Main objective of present study to evaluate antineoplastic activity of ethanolic leave extract of *Tylophora indica* with 7,12 dimethylbenza [a]anthracene (DMBA) as a inducer for skin tumor in mice. **Material and Methods:** Skin tumors were induced in Swiss albino mice by a single topical application of 7,12-dimethylbenz(a)anthracene (100 mg/100 mL acetone) and, 2week later, promoted by repeated applications of croton oil (thrice in a week in 1% acetone) till the end of the experiment (i.e., 16 week). five groups of six Swiss albino mice in each group were used. First group treated with the carcinogen alone second group treated with the carcinogen with standard drug CP(50mg/kg) and Mice of third, fourth, and fifth group were treated with carcinogen along with the ethanolic extract of *Tylophora indica*. at 100 mg/kg, 200mg/kg and 400mg/kg was orally administered. **Result:** *Tylophora indica* extract treatment caused a significant reduction in tumor incidence, tumor yield, and tumor burden, as compared to the 7, 12-dimethylbenz(a)anthracene croton oil-treated control group. **Conclusion:** These results suggest that *Tylophora indica* extract has the potential to become a antitneoplastic agent that can reduce skin cancer in mammals.

KEYWORD: skin carcinogenesis, DMBA, Croton oil, *Tylophora indica.*

INTRODUCTION

Skin cancer is the uncontrolled growth of abnormal skin cells. It occurs when unrepaired DNA damage to skin cells which is most often caused by ultraviolet radiation from sunshine triggers mutations, or genetic defects, leads to the skin cells to multiply rapidly and form

malignant tumors.[1] Although UV radiation is the leading cause of skin cancer, other causative agents include viruses, mutagens in food, mutagens in chemicals and genetic susceptibility[2] DMBA (7, 12-dimethylbenz(a) anthracene) is a polycyclic aromatic hydrocarbon, acts as a pro-carcinogen and is an ultimate carcinogen after metabolic activation. It is widely used as an initiator as well as a promoter to induce skin carcinogenesis in rodents. Therefore, DMBA is commonly employed to study the chemopreventive potential of natural and synthetic entities.[3]

Tylophora indica(Family*: Asclepidaceae*) commonly known as Antmul is a twining perennial herb. The plant is found growing normally in Uttar Pradesh, Bengal, Assam, Orissa, Himalayas and sub Himalayas in India. It also met within Eastern, North- East and Central India, Bengal and parts of South India.[4] Traditionally used for the treatment of jaundice, inflammation and bronchial asthma. Phytochemically, it contains alkaloids, glycoside, tannine, protein, amino acids and is a rich source of flavonoids.[5,6] Epidemiological and experimental studies indicate that phytochemicals from *Tylophora indica* have antioxidative and anti neoplastic properties, which can inhibit tumor initiation, promotion and progression. However, to the best of our knowledge there is no scientific report available on the antineoplastic potential of *ethanolic extract of Tylophora indica(EETI)* till now.

Therefore, the present study was undertaken to examine the antineoplastic potential of EETI against DMBA induced skin carcinogenesis in *Swiss* mice In the present morphological parameter of skin tumor suggest that there is a higher tumor incidence in carcinogen control group as compare to the standard cyclophosphamide and experimental exract of different concentration of *Tylophora indica.*

MATERIAL AND METHOD

Chemicals and reagent

Cyclophosphamide (50 mg/kg) in tablet formulation, it is used for treatment of cancer**.** The initiator 7,12-dimethylbenz(a)anthracene (DMBA) and the promoter croton oil were procured from Sigma Chemicals Co. (St Louis, MO, USA). DMBA was dissolved at a concentration of 100 mg/ 100 mL in acetone. Croton oil was mixed in acetone to give a solution of 1% dilution.

Plant material

Leaves of *Tylophora indica* were collected from the medicinal garden of Amravati, Dist-Amravati January 2017. The plant was identified by Taxonomist.

Extraction Procedure

A weigh quantitiy (500gm) of the coarse powder was taken and extracted with ethanol (90%) in a soxhelet extraction apparatus. The extract was concentrated on a water bath at a temperature not exceeding 60°C the percentage yield of extract was 10% and the ethanolic extract was suspended in distilled water.[7]

Animals

Swiss albino mice (7-8 weeks old weighing 25-27 g), selected from a random breed inbred colony. Which are obtained from the animal house of department of Pharmacology, Vidyabharati college of pharmacy, Amravati. All the animal were acclimatized to the animal house prior to use. They are kept in animal house with a 12 hr light; 12 h dark cycle. The care and handling of mice were in accordance with the internationally accepted standard guidelines for use of animals (CPCSEA)Permission and approval for animal studies was obtained from Institutional Ethics Committee(IAEC) of Vidyabharati college of pharmacy, Amravati SGB Amravati University.

Oral Acute Toxicity of EETI

The animals were fasted overnight prior to the experiment. Different graded doses of *EETI* were administered orally to animal groups and followed for mortality as per the OECD Guideline 425. The LD50 of *EETI* was found at 250 mg/kg body weight. For evaluation of dose related antineoplastic activity, the graded doses of *EETI* viz. 100 mg/kg, 200 mg/kg and 400 mg/kg body weight were chosen.[8]

Experimental Protocol for DMBA-Induced Skin Carcinogenesis Model

The inhibition of tumor development by *Tylophora indica* was evaluated on two-stage process of skin carcinogenesis, induced by a single application of 7,12-dimethylbenz(a) anthracene(initiator) and two weeks later,promoted by repeated application of croton oil (promoter)thrice weekly, using the following protocol for 16 weeks. *Swiss* mice (30) were selected by randomization and divided into five groups of 6 mice each. shaving cream was applied on the intra scapular region of the mice, three days before the commencement of the experiment. Animals for this study were divided into the following groups.

Treatment group

Group I: (Carcinogen-treated control group): Mice (6) belonging to this group were treated with a single topical application of 100 µL DMBA (100 µg/100 µL acetone) over the shaven area of the skin. Two weeks later, 100 µL croton oil (1% w/v in acetone) was applied topically three times per week for 16 weeks.

Group II: (Carcinogen+Standard drug): Animals received oral administration of Cyclophosphamide(40 mg/kg body weight) starting from the time of croton oil application till the end of the experiment (16 weeks). DMBA was given as same in Group I.

Group III: (Carcinogen+low dose of extract): Animals received oral administration of *Tylophora indica* extract (100 mg/kg body weight) starting from the time of croton oil application till the end of the experiment (16 weeks). DMBA was given as same in Group I.

Group IV: (Carcinogen+moderate dose of extract): Animals received oral administration of *Tylophora indica* extract (200 mg/kg body weight) starting from the time of croton oil application till the end of the experiment (16 weeks). DMBA was given as same in Group I.

Group V: (Carcinogen+High dose of extract): Animals received oral administration of *Tylophora indica* extract (400 mg/kg body weight) starting from the time of croton oil application till the end of the experiment (16 weeks). DMBA was given as same in Group I.

Morphological study

pappilomas appearing on the shaven area of the skin were examined at weekly intervals in all the groups. Only those papillomas that persisted more than one week or more, with a diameter greater than 1 mm, were taken into consideration for final evaluation. Skin papillomas that regressed after one week observation were not considered for counting.

Weight	The weight of each tumor was measured at the termination of experiment
Tumor yield	The average number of tumors per mouse was calculated
Tumor burden	The average number of tumors per tumor-bearing mouse was estimated
Tumor incidence	The number of mice carrying at least one tumor was expressed as a percentage incidence

Statistical Analysis

Data from different experimental groups were analyzed and expressed as mean ± standard deviation. Result were analyzed statistically by one way ANOVA followed by DUNNETT's multiple comparison test using graph pad prism and graph pad instat version 3 soft ware,the difference was considered significant if $P<0.05$.

RESULT

Preliminary Phytochemical Screening[9]

Phytochemical test of *Tylophora indica* revealed the presence of alkaloid phenols, saponin, flavonoide, glycoside.

Acute Toxicity Studies

Acute toxicity studies (OECD – 425 guideline) of *Tylophora indica*. revealed that there was no toxic effect up to dose of 2,000 mg/kg nor any significant variation in behavior of animal was observed.

Table 1: Antineoplastic potential of ethanolic extract of Tylophora indica (Burm. f.) on mice skin cancer.

Sr. No.	Groups	Treatment	Body Weight	Tumor Yield	Tumor Burden	Tumor Incidence
1	Group- 1	Carcinogen treated-Control group	24.5±0.22	5.5±0.22	5.6±0.21	100%
2	Group- 2	Standard drug (CP 50mg/kg Body wt.)	31.33±0.33**	0.33±0.21**	1	16.6%
3	Group- 3	EETI (100mg/kg Body wt.)	28.83±0.30**	2±0.25**	4.5±0.22**	50%
4	Group- 4	EETI (200mg/kg Body wt.)	29.66±0.21**	0.66±0.21**	4.16±0.16**	66.6%
5	Group–5	EETI (400mg/kg Body wt.)	30.5±0.22**	0.5±0.22**	2.83±0.16**	33.33%

Each value represents the mean ± S.E.M (n=6). *p<0.05, **p<0.01 compared with control.

[CP: cyclophosphamide EETI: Ethanolic leave extract of Tylophora indica]

1. Body weight

Effect of Ethanolic Extract of *Tylophora indica* on Body weight.

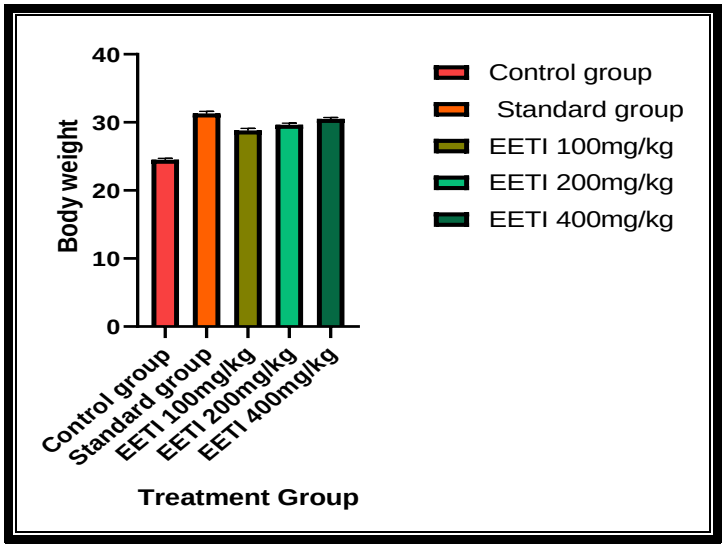

Fig 1: Effect of Ethanolic Extract of *Tylophora indica* on Body weight.

Each value represents the mean ± S.E.M (n=6). *p<0.05, **p<0.01 compared with control.

2)Tumor yield

Effect of Ethanolic Extract of *Tylophora indica* on Tumor yield

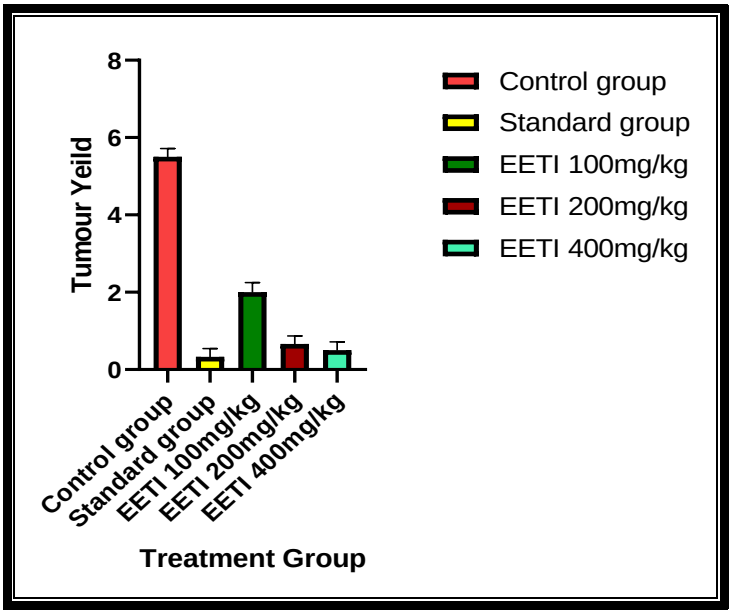

Fig 2: Effect of Ethanolic Extract of *Tylophora indica* on Tumor yield.

Each value represents the mean ± S.E.M (n=6). *p<0.05, **p<0.01 compared with control

3)Tumor Burden

Effect of Ethanolic Extract of *Tylophora indica* on Tumor Burden.

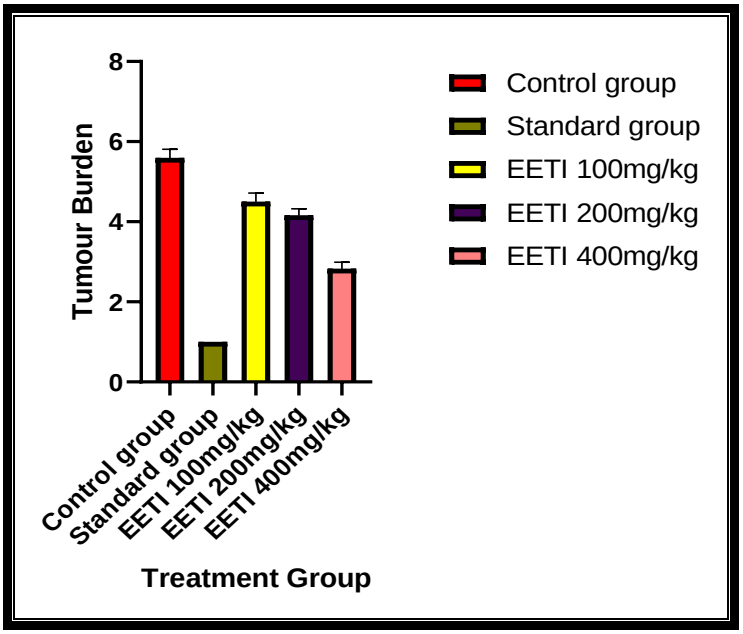

Fig 3: Effect of Ethanolic Extract of *Tylophora indica* on Tumor Burden.

Each value represents the mean ± S.E.M (n=6). *p<0.05, **p<0.01 compared with control.

4) Tumor incidence

Effect of Ethanolic Extract of *Tylophora indica* on Tumor Incidence.

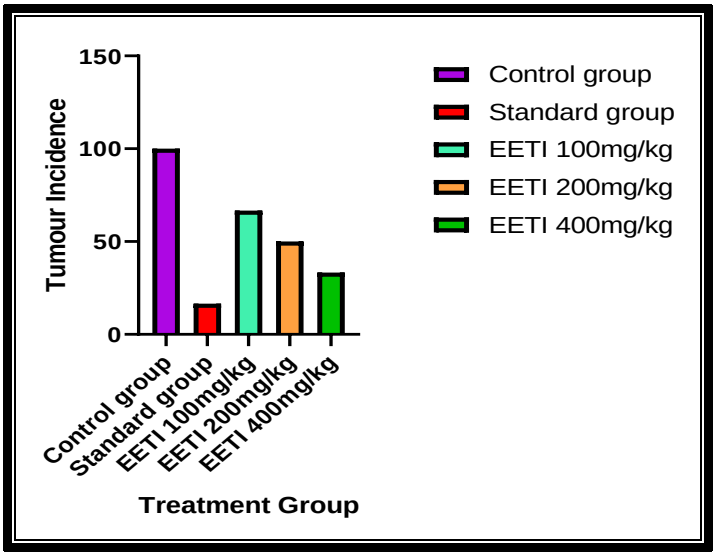

Fig 4: Effect of Ethanolic Extract of *Tylophora indica* on Tumor Incidence.

Each value represents the mean ± S.E.M (n=6). *p<0.05, **p<0.01 compared with control.

DISCUSSION

Skin is a shield that protects people from heat or cold, chemicals, UV-radiation and bacteria. Skin cancer is one of the most common of all human cancers and its incidence is increasing rapidly all over the world. Skin cancer contributes approximately 30% of all newly diagnosed cancer in the world and solar ultraviolet radiation is an established cause of approximately 90% of all skin cancers.[10]

7,12-dimethylbenz(a)anthracene (DMBA), the site and organ specific carcinogen, is commonly employed to induce skin cancer in Swiss albino mice. DMBA could either be used as an initiator or promoter for inducing skin carcinogenesis.[11] The present study discuss the pharmacological effects of *Tylophora indica* as candidate for anticancer activity. Phytochemical studies revealed the presence of compounds especially alkaloids and flavanoids. The significance of phytochemicals alkaloids and flavaniods for treatment of different cancer cells are previosuly described by other workers.[12] The current study demonstrates a antineoplastic potential of *Tylophora indica* extract for DMBA-induced skin tumorigensis in male Swiss albino mice. Animals treated with carcinogen alone showed 100% tumor incidence, high tumor yield, and high tumor burden, due to their carcinogenic potential in the absence of any treatment.

CONCLUSION

From the present study, it is concluded that, *Tylophora indica* the Indian medicinal plant, is a source of many antineoplastic agents and antioxidants Hence the ethanolic extract of *Tylophora indica* has the potential to become a antitneoplastic agent that can reduce skin cancer in mammals.

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