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Study Of Anticancer Activity Of Genistein

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Abstract

Dietary polyphenols are most essential and widely studied due to their various biological activities like anti-cancerous, antibacterial, anti inflammatory, anti diabetic etc. Muramidase is a universal antimicrobial polypeptide which is found in various types of birds, mammals and insects having various pharmaceutical and pharmacological properties like anti-viral, antihistamic, anti- inflammatory, immune modulatory properties found in various types of birds, mammals and insects. It is also known that the enzyme exhibits strong anti-proliferative properties in the form of self-assembled nanostructure particles. Lysozyme has the capacity to cure various diseases by binding with various drugs and realizing them in their target site. The results of the current investigation using the skin tumour model demonstrated that a single topical administration of DMBA followed by croton oil resulted in the development of skin papillomas, which first appeared around the sixth week. By the end of the trial, the mice treated with DMBA + croton oil had a 100% tumour incidence. The total number of papillomas found in these mice was 20.These were significantly reduced in the group which received the treatment of Genistein Test Compound additionally at the dose of 50 and 100 mg/kg body weight as compared with the DMBA + croton oil group. The tumour occurrences dropped to 50% & 66% animals and the values of cumulative number of papilomas in these groups were observed 11 & 8. The average number of papillomas per mouse as well as the papillomas per papilloma-bearing mice was found to be 1.8, 1.3 and 2.7, 2.6. The average latency period was also greater with Genistein Test Compound by topical application. When the discrepancies between the experimental group 2 data were statistically examined ($p < 0.05$), it was discovered that they were significantly different from the control group.

Key word: Genistein, Isoflavones, Lysozyme, Molecular Docking,

INTRODUCTION

Polyphenols are naturally occurring compounds. These are found in the fruits, vegetables, cereals and beverages. There are approximately 200-300 mg polyphenols in 100 g of fresh weight. fruits such cherries, grapes, apples, pears, and berries. A cup of tea or coffee, or a glass of red wine, contain about 100 mg of polyphenols[1, 2].Polyphenols are secondary metabolites of plants which are involved in defense against UV radiation or pathogens [3]. The physical properties like bitterness, astringency, colour, odour, flavour and chemical properties like oxidative stability in food are contributed by polyphenols. Polyphenols have anti-cancers, antidiabetes, anti-inflamatory properties [4, 5]. Polyphenols are so important due to their possible beneficial effects on human health.The largest family and most studied group of polyphenols is flavonoids, which is devided into six catagories (on the basis of number and arrangement of the hydroxyl groups and their extent of alkylation and/or glycosylation) i.e. flavonols, flavones, flavanones, flavanols, anthocyanins and isoflavones. Isoflavones are 3-phenylchromen-4-one, the most essential group of flavonoids which have two bands in UV visible spectroscopy. The 1st bend is observed at 240-280 nm due to benzoyl moiety and 2nd band is due to cinnamoyl moiety at 300-400nm [6].

Mainly genistein and diadzein are found in soybeans. The biological properties of isoflavones include anti-inflammatory, anti-diabetic, anti-cancer, antibacterial, and antitumor activity etc.Genistein (4', 5, 7trihydroxyisoflavone) is a phytoestrogen having a wide variety of pharmacological effects in animal cells. Dietary genistein ingestion has been linked with a range of potential beneficial health effects; especially it has the effect on breast cancer attributed to its moderate binding affinities to estrogen on various tissues.

On human cancer cell lines the effect of polyphenols is most often induce a decrease in the tumour count. [7] At various sites including mouth, stomach, duodenum, colon, liver, lung, mammary gland or skin these effects have been observed. The role of polyphenols has been showed protective effects in some models in different mechanisms [8]. The multistage process of carcinogenesis consists of the three key stages of initiation, promotion, and progression.Initiation is a heritable aberration of a cell. Cells so initiated can undergo transformation to malignancy if promotion and progression follow. Promotion, on the other hand, is affected by factors that do not alter DNA sequences and involves the selection and clonal expansion of initiated cells. Multiple mechanisms of action, including estrogenic/antiestrogenic activity, anti-proliferation, induction of cell cycle arrest or apoptosis, prevention of oxidation, induction of detoxification enzymes, regulation of the host immune system, anti-inflammatory activity, and changes in cellular signalling, have been identified for the chemoprevention effect of polyphenols[9].

The metabolism of pro-carcinogens influence by polyphenols by modulating the expression of cytochrome P450 enzymes involved in their activation to carcinogens. Excretion by increasing the expression of phase II conjugating enzymes is facilitated by them. This induction of phase II enzymes may have its origin in the toxicity of polyphenols [1]. Polyphenols, which are substrates for these enzymes in the body, can result in the formation of toxic quinones. The activation of these enzymes for their own detoxication is due to intake of polyphenols and thus induce a general boosting of our defenses against toxic xenobiotics [10]. It has been demonstrated that tea catechins in the form of capsules when given to men with high-grade prostate intraepithelial neoplasia (PIN) demonstrated cancer preventive activity by inhibiting the conversion of high grade PIN lesions to cancer [11].

MATERIALS AND METHODS

Anticancer Activity of Genistein(Skin Tumour Model)

Animals:

The investigation was done on random bred, 6-7 weeks old and 25 $\pm$ 2 gm body weight of, male Swiss albino mice. These were maintained under controlled conditions of temperature (25$\pm$2˚C) and light (12 light: 12 dark). They were housed in good laboratory condition and were given standard mouse pellet diet and water ad Libitum. One day before the initiation of the experiment, hair on the interscapular region of the mice was removed using hair removing cream in 1 cm^2 area.

Procedure

The procedure described by Berenblum (1975), Sukumaran, and Kuttan was followed during the experiments (1991). A group of six mice (whose dorsal skin had been shaved two days before) were initiated by the single application of 104 µg of DMBA in acetone (100 µl), the croton oil and B. variegata extract was began 1 week after imitationGenistein (100 µl) were applied 1 hrs before each croton oil treatment. The extract was applied to the shaved area using the micropipette. The investigation was continued for 16 weeks. The skin tumours formation were recorded weekly and the tumours greater

than 1 mm in diameter were included in counting of total number of papillomas/mouse, tumor incidence and tumor yield if they persisted two weeks or more.

Experimental Design

The animals were split up into 8 different groups for each extract as follows:
Total no. of animals for each group: - 6 mice
Genistein Test Compound: - 50 mg/kg body weight and 100 mg/kg body weight.
Route - topically

Groups of Genistein (Test Compound)

Group 1 (Vehicle control): 100 µl acetone 2 times /week up to 16 weeks
Group 2 (DMBA alone): 104 µg DMBA was dissolved in 100 µl acetone and single application was given.
Group 3 (Croton oil alone):Up to 16 weeks, 1% Croton oil was applied to the skin twice a week. Group 4 (Genistein alone): 100 µl was given 2 times in a week up to 16 weeks.
Group 5 (DMBA + Croton Oil):After dissolving 104 µg of DMBA in 100 µl of acetone, a single application was administered. Up to 16 weeks, 1% Croton oil was applied to the skin twice each week.
Group 6 (DMBA + Genistein (Lower Dose) + Croton Oil):A single application of 1% croton oil was applied after 104 µg of DMBA had been dissolved in 100 µl of acetone. One hour earlier, 100 µl of Genistein at a dose of 50 mg/kg had been administered. This procedure was repeated twice weekly for a total of 16 weeks.
Group 7 (DMBA + Genistein (Higher Dose)+ Croton Oil): 104 µg DMBA was dissolved in 100 µl acetone and single application was given afterwards the 100 µl dose of Genistein at the dose of 100 mg/kg was given one hour before the each application of 1 % croton oil, 2 times in a week up to 16 weeks.

Study parameters

- Cumulative number of Papillomas
- Tumor incidence
- Tumor Yield
- Tumour bardon

Statistical analysis

The differences of the tumors among different groups were considered to be significant at 5% significance level ($p<0.05$) when evaluated by Student's 't' test.

RESULTS AND DISCUSSION

ANTICARCINONENIC STUDIES:

Anticarcinogenicity activity of Genistein Test Compound:

The results of the current investigation using the skin tumour model demonstrated that a single topical administration of DMBA followed by croton oil resulted in the development of skin papillomas, which first appeared around the sixth week. By the end of the trial, the DMBA + croton oil treated mice (carcinogen control) had a 100% tumour incidence (16 weeks). The total number of papillomas in these mice was 20. These were significantly reduced in the group which received the treatment of Genistein Test Compound additionally at the dose of 50 and 100 mg/kg body weight (Grp. VI, and VII) as compared with the DMBA + croton oil group (Grp. V). The incidences of tumours were decreased to

50 & 66% animals and the values of cumulative number of papilomas in these groups were observed 11 & 8. It was found out that the average papilloma yield and burden per papilloma-bearing mouse were 1.8, 1.3 and 2.7, 2.6, respectively.The average latency period (i.e. time lag between the application of the promoter and the appearance of 50% of tumors) was also greater with Genistein Test Compound by topical application. The differences in the values of the results of different experimental groups were statistically analyzed ($p< 0.05$) and found to be significant in comparison to the control group (Grp. V).The Genistein Test Compound alone, No therapy, Croton oil alone, and DMBA alone groups did not cause any tumour incidence.Each group consists of 6 animals. Table 1 provides a summary of the findings.

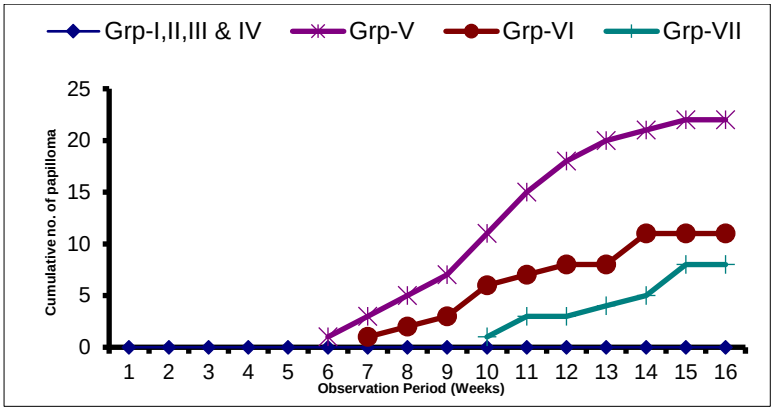
Table 1. Effect of Genistein Test Compound on DMBA-induced papillomas in Swiss albino mice

Sl	Groups	Body weight (Mean ± SE)		Cumulative No. of Papillomas	Tumour ncidence	Tumour Yield	Tumour Burden
		Initial	Final				
I	Vehicle alone	25.1 ± 0.44	29.6±0.22	0	0/6	0	0
II	DMBA alone (1 application)	26.1± 0.24	31.1±0.39	0	0/6	0	0
III	Croton oil alone	24.9± 0.78	29.1±0.45	0	0/6	0	0
IV	Genistein Test Compound alone	25.2± 0.12	30.1±0.77	0	00	0	0
V	DMBA+ Croton oil	26.6± 0.25	31.2±0.55	20	6/6 (100%)	3.33±0.78	3.33±0.35
VI	DMBA + Genistein Test Compound (50 mg/kg)+ Croton oil	25.7± 0.64	29.6±0.34	11	4/6 (66.66%)	1.8±0.65*	2.7±0.47*
VII	DMBA+ Genistein Test Compound	26.8±0.21	31.4±0.77	8	3/6	1.3±0.61*	2.6±0.19*

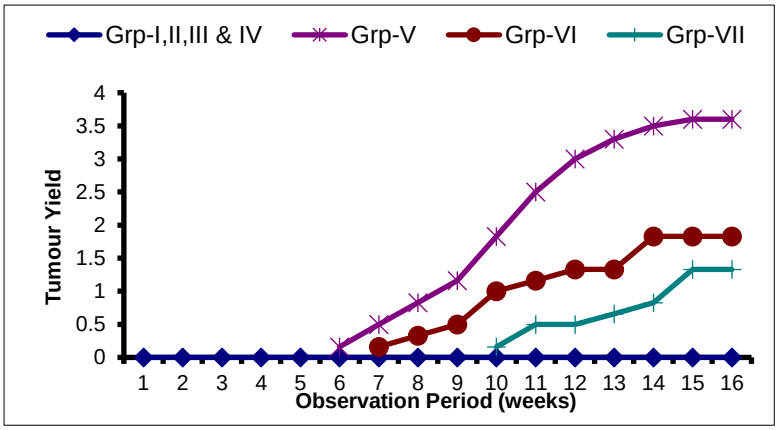
	(100 mg/kg)+ Croton oil				(50 %)		

*Significance level among different groups at p< 0.05. Carcinogen control v/s Genistein Test Compound experimental.

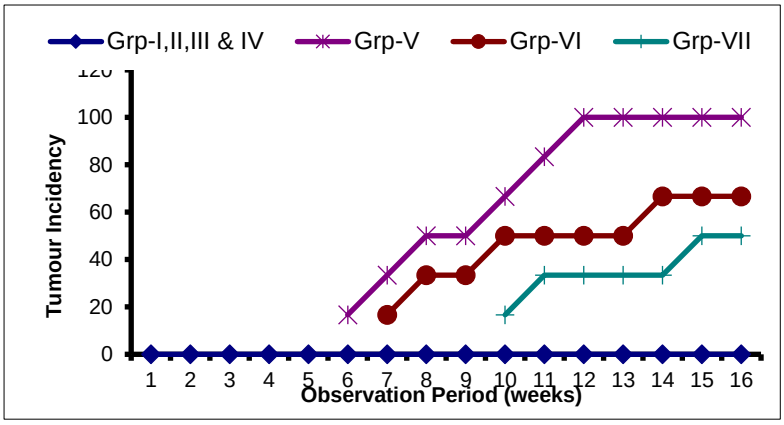
Single application of DMBA was given at the dose of 104 mg/kg body wt. and 1 week later 1% croton oil was given 1 hour after each applications of Genistein Test Compound 2 times/week until the end of the experiment (i.e. 16 weeks); Genistein Test Compound 50 and 100 mg/kg/2 cm2/week/mice treatment starting with the application of DMBA until the end of the experiment.



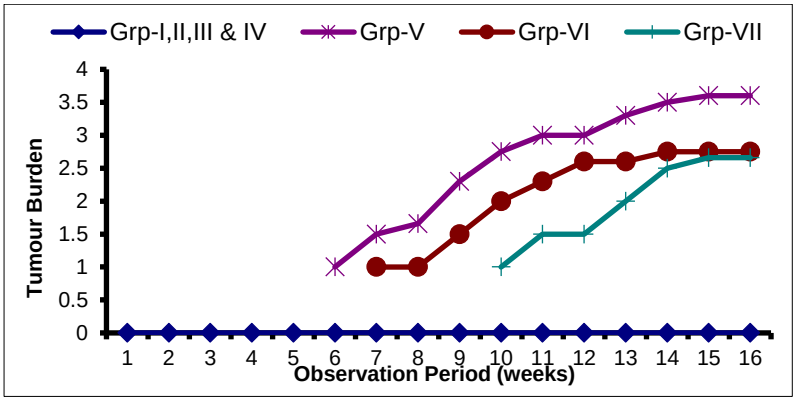
Graph 3 Effect of Genistein Test Compound on cumulative number of papilloma



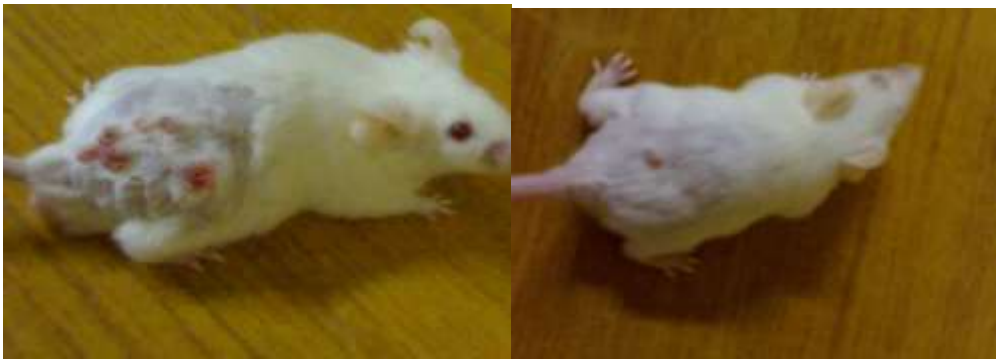
Graph 4 Effect of Genistein Test Compound on Tumour yield of papilloma



Graph 5 Effect of Genistein Test Compounon Tumour Incidency



Graph 6 Effect of Genistein Test Compounon Tumour Burden



A) B)

A) Photograph showing the skin tumour induced by DMBA + Croton oil for 16 weeks, B) Photograph showing the reduced skin tumour which received the treatment of DMBA + Genistein + Croton oil for 16 weeks

CONCLUSION

The results of the current investigation using the skin tumour model demonstrated that a single topical administration of DMBA followed by croton oil resulted in the development of skin papillomas, which first appeared around the sixth week. By the end of the trial, the DMBA + croton oil treated mice (carcinogen control) had a 100% tumour incidence (16 weeks). The cumulative number of papillomas in these mice was recorded as 20. These were significantly reduced in the group which received the treatment of Genistein test compound additionally at the dose of 50 and 100 mg/kg body weight (Grp. VI, and VII) as compared with the DMBA + croton oil group (Grp. V). The incidences of tumours were decreased to 50 & 66% animals and the values of cumulative number of papilomas in these groups were observed 11 & 8. It was discovered that the average papilloma yield and burden per papilloma-bearing mouse were 1.8, 1.3 and 2.7, 2.6, respectively.The average latency period was also greater with Genistein Test Compound by topical application. When the discrepancies between the experimental groups' results were statistically assessed ($p< 0.05$), it was discovered that they were

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