Antimutagenic and wound healing activity of *Emblica officinalis* extract in Swiss Albino mice

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Abstract

Single application of Emblica ext. at the dose of 50 ,100 and 150 mg/kg body weight ,24hours prior the i.p. administration of Cyclophosphamide (at the dose of 50 mg/kg) have significantly prevented the micronucleus formations and chromosomal aberrations in dose dependent manner in bone marrow cells of mice as compared to Cyclophosphamide group. However, Emblica ext alone has not induced any micronucleus formations and chromosomal aberrations in bone marrow cells as compared to control group. In another experiment, topical application of Emblica extract at the dose of 500 mg/kg b.wt. have shown wound healing activity on the skin of Swiss mice. The wound created by excision method was almost healed after 21 days but in 8-15 days the wound healing by Emblica extract was greater than Betadine treated group (Positive control). The above studies showed the Chemopreventive potential of Embelica extract which is an important plant used in Aruvedic preparations of medicine.

Introduction

Emblica officinalis is one of the most important plants in the traditional medicine system of India. Emblica officinalis (Family: Euphorbiaceae) is valued for its unique tannins and flavanoids, which are considered to contain very powerful antioxidant compounds. There are several reports which showed the antioxidant properties of amla and its constituents, Emblicanin A and B in experimental animals [1-3] and also reported to inhibit lipid peroxidation and reduction of blood sugar levels [4,5]

A simple aqueous extract of *P. emblica* fruit was shown to protect mice against the chromosome damaging effects of the well known carcinogen 3,4-benzo(a)pyrene.[6] and protected mice against the effects of nickel chloride [7]. It also prevented radiation-induced DNA strand breaks in a dose dependent manner.

The radical scavenging capacity of EOE could be attributed to its constituent phenolics [8] .An anti-tumour effect of *P. emblica* aqueous fruit extract was also demonstrated in tumour-bearing mice, resulting in a 35%increase in life span of experimental animals [9] and meditated primarily through enhanced natural killer cell activity and antibody-dependent cellular cytotoxicity [10]

An aqueous extract of P. Emblica have significantly reduced induced solid tumours in mice in a manner suggesting interaction with cell cycle regulation [11] , and also inhibited the proliferation of four human tumour cell lines in vitro [12]. E.O and chyavanaprash (a non-toxic herbal preparation containing 50% E.O extracts were found to reduce ascites and solid tumours in mice induced by DLA cells[13]. It was also reported the chemopreventive potential of Emblica officinalis fruit extract (500 mg/kg) on DMBA induced skin tumorigenesis in Swiss albino mice [14] and could decrease the liver tumour induced by NDEA [15] .EO extracts were reported to inhibit the hepatotoxicity produced by acute and chronic CCl_4 administration.[16] and significantly inhibited hepatocarcinogenesis induced by N-nitrosodiethylamine (NDEA) in a dose dependent manner. Reduction was seen in tissue levels of reduced glutathione. Serum

levels of lipid peroxide (LPO), alkaline phosphatase (ALP) and glutamate pyruvate transaminase (OPT), which are markers of liver injury, were also elevated [17].

An extract of P. Emblica fruit and the flavonoid constituent quercetin were shown to provide significant protection against liver toxicity caused by ethanol and paracetamol in vivo. [18]

An aqueous extract of P. Emblica fruit protected mice against the hepatotoxic and nephrotoxic effects of lead and aluminum salts. [19] The fruit of P. Emblica have shown to contain a number of compounds with potent inhibitory activity against human immunodeficiency virus (HIV) reverse transcriptase. [20]

Amla significantly inhibited Cr-induced free radical production and restored the anti-oxidant status back to control level. Increased glutathione peroxidase (GPx) activity and diminished glutathione (GSH) levels. [21] Amla is an important herbal compound and there is limited report about the Chemopreventive activity, it is therefore, we have undertaken to study the antimutagenic activity using micronucleus and chromosomal aberration test and inhibition of wound healing activity in Swiss albino mice.

Materials and Methods

Chemicals *Emblica officinal* powder (Amla) was obtained from the local Aruvedic shop and 50 % methanolic extract was prepared. Cyclophosphamide was purchased from Sigma chemical Co., U.S.A. and other chemical were reagents grade and were procured locally for the study.

Micronucleus Assay and chromosomal aberrations test

Animals and Treatment: Male Swiss albino mice of 15-20 g body wt. were obtained from the animal colony of our Research Centre and 4-5 animals were housed in each plastic cage. The animals were provided standard pallet diet and water ad libitum.

The extract at the volume of 0.2 ml was injected 24 hours before the treatment. The positive control group received single i.p. injection of 50 mg/kg cyclophosphamide in 0.9% saline. Colchicine (4 mg/kg b.wt) was administered intraperitoneally 2 hours before the harvest of

the cells. Animals were sacrificed by cervical dislocation and bone marrow cells were harvested. The slides were prepared essentially as per modified method of Preston et al (1987) for chromosomal aberrations and method of Schmidt (1975) for micronucleus evaluations. For chromosomal aberration assay, the femur was excised and the bone marrow was extracted in 0.56 % KCl. The harvested cells were incubated at 37^0C for 20 minutes and then centrifuged for 10 minutes at 1000 rpm . Cells were fixed in Carnoy's fixative (Methanol: Acetic acid, (3:1) and burst opened on a clean slide to release the chromosomes. The slides were stained with 5 % Giemsa solution for 15 minutes and then put in xylene and mounted with DPX. A total of 100 well spread metaphase plates were scored for chromosomal aberrations at a magnification of 1000 X (100 x 10 X) for each group. Different types of chromosomal aberrations such as chromatid breaks, gaps, pulverization, polyploidy, centromeric association etc. were scored and expressed as % chromosomal aberrations. About 1000 cells were counted and number of micronucleated cells were also scored. The data are presented in MNPCE+SE. The statistical significance was evaluated using Student's `t' test .

Wound healing Methods

Excision wound models were used to evaluate the wound healing activity of Amla extracts on Swiss Albino mice . In this model, animals were divided into three groups of 6 animals each. In, group 1 served as control and group 2 as reference standard. In group 3 animals were treated with *Amla extract* (500 mg kg^{-1} day^{-1}) for 14 days respectively. The effects of vehicles on the rate of wound healing were assessed by the rate of wound closure, period of epithelialisation, and histopathology of the granulation tissue.

Results

Single application of Emblica ext at the dose of 50 ,100 and 150 mg/kg dry weight ,24hours prior the i.p.administration of Cyclophosphamide (at the dose of 50 mg/kg) have prevented the

micronucleus formations in dose dependent manner in bone marrow cells of mice as compared to Cyclophosphamide group. However, Emblica extract alone has not induced any micronucleus formations in bone marrow cells as compared to control group (Table 1)

Single application of Emblica ext at the dose of 50 ,100 and 150 mg/kg dry weight ,24hours prior the i.p.administration of Cyclophosphamide (at the dose of 50 mg/kg) have also prevented the chromosomal aberrations in dose dependent manner in bone marrow cells of mice as compared to Cyclophosphamide group. However, Emblica extract alone has not induced any chromosomal aberrations in bone marrow cells as compared to control group (Table 2)

Topical application of Emblica extract at the dose of 500 mg/kg b.wt. have shown wound healing activity on the skin of Swiss mice. The wound created by excision method was almost healed after 21 days but in 8-15 days the wound healing by Emblica extract was greater than Betadine treated group (Positive control). The histopathology studies have shown formation of epithelial and connective tissue after 7 days (Table 3)

Discussion

In the present investigations , 50 % methanolic extract of . *Emblica* fruit was shown to protect mice against the chromosome damaging effects of the well known mutagen cyclophosphamide. Single application of Emblica extract. at the dose dependent manner have prevented the micronucleus formations in bone marrow cells of mice.

The antioxidant properties of amla and its constituents have been reorted (1-3) Amla significantly inhibited Cr-induced free radical production and restored the anti-oxidant status back to control level. It also increased glutathione peroxidase (GPx) activity and diminished glutathione (GSH) levels. [13] It is possible that chemoprotective activity of EOE could be attributed to its hydroxyl and superoxide radicals scavenging property along with its

lymphoproliferative activity. The radical scavenging capacity of EOE could be attributed to its constituent phenolics. [8]. The above studies showed the Chemopreventive potential of Embelica extract which is an important plant used in Aruvedic preparations of medicine.

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Table 1

Effect of E.O. on micronucleus formation in Bone marrow cells of mice.

Treatment	MNPCE± SE	PCE/NCE Ratio
1 EO + CY (50 mg/kg) +(50 mg/kg)	1.33 ± 0.3	1.059 ± 0.161
2 EO + CY (100 mg/kg) +(50 mg/kg)	1.166 ± 0.81	0.602 ± 0.385
3 EO + CY (150 mg/kg) +(50 mg/kg)	0.75 ± 0.49	0.357 ± 0.167
4 EO Alone(50 mg/kg)	0.75 ± 0.49	0.859 ± 0.166
Positive control 5 Cyclophosphamide Alone	2 ± 0.816	0.693 ± 0.106

Effect of E.O. on chromosomal aberrations in Bone marrow cells of mice.

Dose	*% chromosomal aberrations*	*Chro. Association*	*Chro. Ring*	*Chro. Gap*	*Chro. Fragment*	*Chro. Break*	*% protection*
1 EO + CY (50 mg/kg) +(50 mg/kg	44.38 ± 30.38	12%	8%	6%	7%	11%	
1 EO + CY (100 mg/kg) +(50 mg/kg	32.01 ± 11.50	17%	2%	4%	5%	7%	
1 EO + CY (150 mg/kg) +(50 mg/kg	31.22 ± 19.89	13%	5%	2%	2%	5%	
EO Alon(50 mg/kg) e	19.80 ± 10.82	9%	4%	1%	Nil	4%	
Cyclophosphamide	66.5 ± 30.54	4%	Nil	8%	32%	4%	
Alone (50 mg/kg)							

Wound healing activity of emblica officinalis extract on Swiss albino mice

Group	*Anterior side (mm)*	Posterior side (mm)
1.Bitadine (POSITIVE CONTROL)		
1-7 Days	52.5 ± 6.75	64 ± 9.55
8-14 Days	23.74 ± 0.20	21.79 ± 23.89
15-21 Days	0.00	0.00
2.Untreated (CONTROL)		
1-7 Days	62.5 ± 6.84	62.2 ± 22.85
8-14 Days	56.2 ± 3.76	44.2 ± 5,3
15-21 Days	9.6 ± 0.7	8.4 ± 1.5
3.Emblica officinalis extract (TEST)		
1-7 Days	40.10 ± 8.03	45.56 ± 10.23
8-14 Days	7.89 ± 4.91	16.17 ± 13.79
15-21 Days	0.14 ± 0.11	0.67 ± 0.58

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