

COMPARATIVE EVALUATION OF ANTICANCER ACTIVITY OF CRUDE EXTRACTS AND ISOLATED COMPOUND SALICIN OF DESMODIUM GANGETICUM (L) DC AGAINST EHRLICH ASCITES CARCINOMA IN SWISS ALBINO MICE

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ABSTRACT

Ethnopharmacological relevance: The plant *Desmodium gangeticum* (L.) DC (Fabaceae), is commonly known as 'shalparni' in India and 'ticktree' in English. Traditionally the whole plant is a bitter tonic used as anti-tumor, anti-inflammatory and anti-ulcer. **Aim of study:** The objective of the present study was to explore the anticancer activity of the methanol (MEDG), ethylacetate (EADG) extracts and isolated compound salicin of the *D. gangeticum* against Swiss albino mice Ehrlich Ascites Carcinoma (EAC) cell line. **Materials and methods:** Anticancer activity of MEDG, EADG extracts and salicin of *D. gangeticum* were evaluated in Swiss albino mice against Ehrlich Ascites Carcinoma (EAC) cell line at the doses of 100 and 200 mg/kg body weight. MEDG and EADG were administered intraperitoneally

for nine consecutive days. Twenty-four hours after the last dose and 18 h of fasting, the mice were sacrificed and antitumor effect of MEDG, EADG and salicin were assessed by evaluating tumor volume, tumor weight, viable and nonviable tumor cell count, hematological parameters and biochemical parameters of the EAC bearing host. **Results:** MEDG, EADG and salicin administration produced significant ($p < 0.01$) decrease in tumor weight, tumor volume, viable cell count and elevated the life span of EAC tumor bearing

mice. Hematological profile, such as RBC, WBC, lymphocyte counts and hemoglobin content reverted to near normal level in MEDG, EADG and salicin treated mice. These extracts significantly ($p < 0.05$) decreased the level of lipid peroxidation and significantly ($p < 0.05$) increased the levels of GSH, SOD and CAT. **Conclusion:** The data indicated that the *D. gangeticum* extracts as well as salicin compound have potent dose dependent anticancer activity, which approaches that of 5-fluorouracil.

KEYWORDS: 5-Fluorouracil, Anticancer activity, *Desmodium gangeticum*, Ehrlich Ascites Carcinoma (EAC) cell line, Ethylacetate extract, Methanol extract, Salicin,

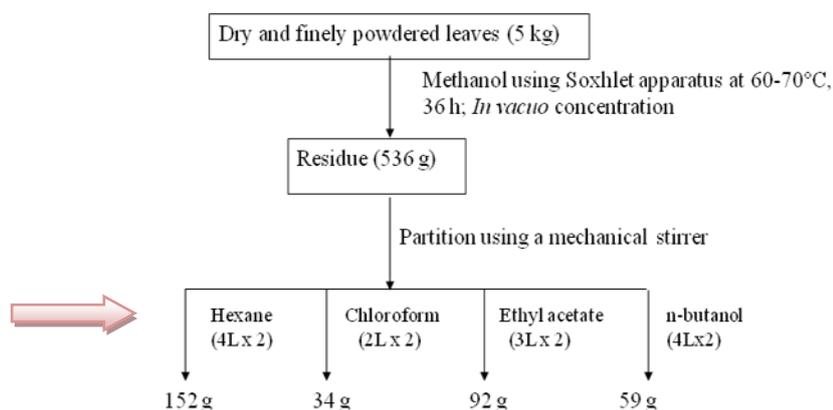
ABBREVIATIONS: MEDG: methanol extract of *Desmodium gangeticum*; EADG: ethylacetate extract of *D. gangeticum*, EAC: Ehrlich Ascites Carcinoma; %ILS: percentage increase in life span; MST: mean survival time; B.W.: body weight; WBC: white blood cell; RBC: red blood cell; Hb: hemoglobin; CRD: Completely Randomized Design; TBARS: Thiobarbituric Acid Reactive Substances

Graphical abstract

D. gangeticum (100 and 200 mg/kg, i.p.) MEDG, EADG extracts and salicin have potent dose dependent anticancer activity. Thus *D. gangeticum* could be useful clinically in the prevention of cancer.



Desmodium gangeticum



Extraction of *Desmodium gangeticum* leaves

Leaf extracts and salicin of *D. gangeticum* showed anticancer activity

INTRODUCTION

Uses in traditional medicine and reported activities

D. gangeticum, commonly known as 'shalparni' or 'Ticktree', is a valuable medicinal plant widely used in Indian traditional medicine 'Ayurveda' for the treatment of various inflammatory conditions of chest and other organs due to vata disorder as well as for ischemic heart disease.^[1-2] The plant shoot and aerial parts extract was reported to contain alkaloids, flavonoid glycosides, lipids, glycolipids, isoflavanoid and sterols N,N-dimethyltryptamine, 5-methoxy-N,N dimethyltryptamine, their oxides while pterocarpenoids are the major constituents of the root.^[3] and plant was expected to possess anticancer activities.^[4] Aminoglucosyl glycerolipid was identified from whole plant extract, and it showed antileishmanial and immunomodulatory activities *in vitro*.^[5]

The plant is used in 'Ayurvedic' preparations like 'Dashmoolarishta' and 'Dashmoola kwaath' for the post-natal care in order to avoid secondary complications and nervous weakness and also for treatment of infections and liver diseases.^[6]

The aqueous extracts of this plant exhibit wound healing, antidiabetic and anti-inflammatory activities.^[7-8] and also showed severe antiwrithing, moderate central nervous system depressant and antileishmanial activities.^[5,9] *D. gangeticum* is expected to have anti-oxidant activities in its aerial parts.^[10] Kurian and Paddikala,^[11] reported that feeding the aqueous extract of *D. gangeticum* improved the antioxidant capacity of heart and reduced the degree of lipid peroxidase after ischemic perfusion. A similar effect was observed when rats were given ethylacetate extract of *D. gangeticum* roots.^[12] Its ethanolic extract act as a potent antiulcer agent in all models.^[13] Feeding 50-200 mg/kg p.o. *D. gangeticum* aqueous extract for seven days significantly improved learning and memory in mice, and it reversed natural ageing and amnesia induced by scopolamine.^[14] Isolation of bioactive compound salicin from leaves of *D. gangeticum* is reported by Srivastava *et al.*^[15]

So far, there has been no systematic study to examine anticancer activity in the leaves of this plant. Therefore, the present study, based on the ethnomedical claims, aimed to evaluate the anticancer activity of methanol, ethylacetate extracts and salicin compound obtained from *D. gangeticum* leaves against EAC tumor model.

MATERIAL AND METHODS

Plant Collection

The plant material *D. gangeticum* (L.) DC (Family: Fabaceae) was collected from Ayurvedic Garden, Institute of Medical Sciences, B.H.U, Varanasi, Uttar Pradesh, India and taxonomically authenticated by Dravyagune, BHU, Varanasi, India. *D. gangeticum* naturally occurs on the lower hills and in the plains throughout India.

Preparation of extract and isolation of salicin

The leaves of *D. gangeticum* were shade dried, powdered, and 5 kg powder was extracted with methanol (6×5 L) at 60-70°C for 36 h using a Soxhlet apparatus. The extract was concentrated to dryness under reduced pressure and controlled temperature (25°C); it yielded 536 g residue, which was further fractionated in n-hexane (2 L×2), chloroform (1 L×1), and EtOAc (1 L×3) using a mechanical stirrer followed by concentration under reduced pressure to afford crude residue of 152g, 34g and 92g, respectively. Systematic chemical investigation of the methanolic leaf extract enabled isolation of known glycoside, 2-(hydroxymethyl) phenyl hexopyranoside (DG-1),^[4,15] also known as 'salicin' which is conventionally isolated from the willow bark.^[16] this is the first report of isolation of salicin from leaves of *D. gangeticum*. The extracts were preserved in a refrigerator at 4°C until further use. Preliminary phytochemical screening indicated the presence of flavonoids, terpenoids, glycosides, tannins and alkaloids in the extracts.

Acute toxicity

As per reported method.^[17] an acute toxicity study relating to the determination of the LD₅₀ value of MEDG and EADG in male Swiss albino mice was determined. Different groups of mice were treated with of MEDG or EADG extracts of leaves as well as salicin (100 mg, 200 mg upto 500 mg) intraperitoneally. One group was maintained as control and was given normal saline. The animals were observed continuously for 2 h, and then intermittently and after 24h for 14 days.

Chemicals

5-Fluorouracil was obtained from Ameresco Laboratories, Ltd., India. The other chemicals used were sodium chloride, propylene glycol, trypan blue, methyl violet, sodium sulphate and methylene blue (Merck Limited, Mumbai, India). All other chemicals and reagents used were of highest analytical grade.

Animals

Swiss albino mice of either sex weighing between (18-22 g) were used for the present study. They were obtained from the central animal house, Institute of Medical Sciences, BHU, India. The mice were grouped and housed in polyacrylic cages (38cm×23cm×10 cm) with not more than eight animals per cage and maintained under standard laboratory conditions (temperature 25±2 °C and dark/light cycle 14/10 h). They were allowed free access to standard dry pellet diet and water ad libitum. All procedures with animals were reviewed and approved by the University Animal Ethical Committee.

Transplantation of tumor

EAC cells were obtained from National Centre for Cell Sciences (NCCS), Pune, India. The EAC cells were maintained in vivo in Swiss albino mice by intraperitoneal transplantation of 2×10^6 cells per mouse after every 10 days. Ascitic fluid was drawn out from EAC tumor bearing mice at the log phase (days 7–8 of tumor bearing) of the tumor cells. Each animal received 0.1 ml of tumor cell suspension containing 2×10^6 tumor cells intraperitoneally.

Treatment schedule

Swiss albino mice were divided into 9 groups (n = 20) and given food and water ad libitum. All the groups were injected with EAC cells (2×10^6 cells/mouse) intraperitoneally except for the normal group. This was taken as day zero. On the first day normal saline (0.85%, w/v, NaCl) 5 ml/kg/mouse/day i.p. served as Group-I and EAC control (without any treatment) served as Group-II. Methanol extract (MEDG) was given at 100 mg/kg body weight/day in Group-III and at 200 mg/kg/day in Group IV, while ethylacetate extract (EADG) was administered at 100 mg/kg/day in Group-V and at 200 mg/kg/day in Group-VI. Salicin at 100 mg/kg body weight/day in Group-VII and at 200 mg/kg/day in Group VIII, and the standard drug 5-Flurouracil (5-FU) at 20 mg/kg/day was injected in Group- IX. After twenty-four hours from the last dose and 18 h of fasting, 10 animals of each group were sacrificed by cervical dislocation to measure antitumor, hematological and biochemical parameters. The rest of the animals of each group were maintained to assess their lifespan, and they were provided food and water ad libitum. The effect of MEDG, EADG and salicin on tumor growth and host's survival time were examined by studying the parameters like tumor volume, tumor cell count, mean survival time, increase in lifespan of EAC bearing mice.

Hematological parameters

At the end of the experimental period, the next day after an overnight fasting blood was collected from freely flowing tail vein and from eye and used for the estimation of hemoglobin (Hb) content.^[18] red blood cell (RBC) count and white blood cell (WBC) count.^[19] and differential count of WBC.^[20]

Biochemical parameters

After the collection of blood samples, the mice were sacrificed by cervical dislocation. The liver was excised, rinsed with ice cold normal saline followed by a rinse with ice-cold 10% KCl solution, blotted dry and weighed. A 10% w/v homogenate was prepared in ice-cold KCl solution and centrifuged at 1500 rpm for 15 min at 4 °C. The supernatant thus obtained was used for the estimation of lipid peroxidation.^[21] glutathione (GSH).^[22] superoxide dismutase (SOD).^[23] and catalase (CAT).^[24]

Tumor volume

The ascitic fluid was collected from the peritoneal cavity, of the animal and its volume was estimated using graduated centrifuge tubes.

Tumor weight

The tumor weight was estimated as the difference between weights of the mice before and after the collection of the ascitic fluid from their peritoneal cavity.

Percentage increase in life span

The effects of MEDG, EADG and salicin on life span were estimated on the basis of mortality of the experimental mice as described by Bala *et al.*^[25]

Tumor cell count

The ascitic fluid was diluted 100 times with normal saline; one drop of this suspension was placed on the Neubauer's counting chamber and the numbers of cells in 64 small squares were counted.

Viable/nonviable tumor cell count

The viability of the cells was detected by checked by staining the cells with trypan blue (0.4% in normal saline) dye. Viable cells do not take up this dye; nonviable cells were take up this stain. The numbers of these viable and nonviable cells were estimated.

Statistical analysis

All the parameters studied were subjected to statistical treatment using SPSS statistical package (Version 18). The data were subjected to ANOVA according to CRD, and the significance of differences between the treatment means were determined by the Duncan's new multiple range test (DMRT) at a $P < 0.05$ level.^[26]

RESULTS

This study was undertaken to evaluate the anticancer activity of the leaf extracts of *D. gangeticum*, to assess its potential for cancer therapy. This plant is commonly used in Ayurvedic system of medicine for managing a variety of disorders. The MEDG, EADG and salicin.^[15] were evaluated for their anti-tumor activity in EAC bearing mice and the results are tabulated in Tables 1- 3.

Effect of MEDG, EADG and salicin on tumor volume and survival time

There were no gross behavioral changes and mortality upto a dose level of 300 mg/kg body weight. The LD₅₀ value of MEDG, EADG extracts and salicin were found to be > 2 g/kg body weight of mice indicating that it has low toxicity to the animal. Treatment with MEDG, EADG extracts and salicin at the dose of 100 and 200 mg/kg body weight increased the lifespan (ILS) and nonviable cell count and significantly reduced the tumor volume, tumor weight and viable tumor cell count when compared to that of EAC control group (Table 1).

The effect of MEDG, EADG and salicin on hematological studies

The haemoglobin content, RBC count, lymphocyte (%) and monocyte (%) in EAC bearing mice given MEDG, EADG extracts and salicin at the dose of 100 and 200 mg/kg increased significantly compared with those in EAC control, whereas WBC count and neutrophil (%) showed significant decrease (Table 2). Treatment with MEDG, EADG extracts and salicin restored the hematological parameters to more or less normal values. The number of RBC count and hemoglobin content also increased, while the WBC and the differential count decreased as compared to that of EAC control. Treatment with MEDG, EADG leaf extracts and salicin illustrated the percent increase in tumor cell volume and numbers of viable tumor cells were found to be significantly less when compared to those of the EAC control. Hence, it can be concluded that the extracts by their cytotoxic effect and by arresting the tumor growth, increased the life span of EAC-bearing mice. The percentage increase in life span in response to the 200 mg/kg body weight of salicin administration was found to be much higher than the MEDG and EADG extracts indicating its potent anticancer nature (Table 2).

In acute toxicity studies, the administration of MEDG, EADG and salicin at the dose of 100 mg/kg and 200 mg/kg for 14 days did not exhibit any adverse effect which may be due its composite nature where the presence of these phyto-constituents could counteract its toxicity.

The effect of MEDG, EADG and salicin on biochemical studies

As shown in Table 3, the level of thio-barbituric acid substances (TBARS) was significantly increased in the EAC treated animals when compared to the normal group. Treatment with MEDG, EADG leaf extracts and salicin at 200 mg/kg body weight reversed these changes towards normal levels. Significant decrease in the levels of GSH, SOD and CAT was observed in EAC control group which was reversed significantly towards normal in the MEDG, EADG and salicin treated groups. Almost similar results were observed with 5-FU treatment.

Table 1: Effect of the methanol extract of *D. gangeticum* plant on tumor volume, tumor weight, mean survival time (MST), percentage increase life span (%ILS), viable and nonviable, tumor cell count in EAC bearing mice

Parameters	EAC control	100 mg/kg MEDG	200 mg/kg MEDG	100 mg/kg EADG	200 mg/kg EADG	100 mg/kg salicin	200 mg/kg salicin	5-FU
Tumor volume (ml)	3.12 ^a	1.98 ^c	1.01 ^d	2.12 ^b	1.52 ^{cde}	1.68 ^{cd}	0.99 ^{de}	0.45 ^e
Tumor weight (g)	3.90 ^a	1.80 ^{bc}	0.99 ^d	1.98 ^b	1.10 ^{cd}	1.52 ^c	0.89 ^{de}	0.40 ^e
MST (days)	21	31	37	28	32	34	40	42
%ILS	0.0	47.6	76.19	33.3	52.38	54.54	81.81	100
Viable cell count	7.9×10^7 ^a	3.4×10^7 ^{bc}	1.6×10^7 ^d	3.9×10^7 ^b	2.1×10^7 ^c	3.1×10^7 ^{bcd}	1.3×10^7 ^{de}	0.92×10^7 ^e
Nonviable cell count	0.7×10^7 ^e	1.5×10^7 ^{de}	3.0×10^7 ^b	2.1×10^7 ^c	2.9×10^7 ^{bc}	1.9×10^7 ^d	2.8×10^7 ^{bcd}	3.7×10^7 ^a
Total cell count	8.6×10^7	4.9×10^7	4.6×10^7	6.0×10^7	5.0×10^7	5.0×10^7	4.8×10^7	4.6×10^7

Each point represents the mean (n = 10 mice per groups).

The values marked with the different letters show significant difference (Duncan's multiple range test, P < 0.05).

Table 2: Effects of methanol and ethyl acetate extract of *D. gangeticum* on hematological parameters of EAC treated mice.

Parameters	Normal Saline (0.5 ml/kg)	EAC (2×10 ⁶ cells) control	EAC (2×10 ⁶ cells) + 100 mg/kg MEDG	EAC (2×10 ⁶ cells) + 200 mg/kg MEDG	EAC (2×10 ⁶ cells) + 100 mg/kg EADG	EAC (2×10 ⁶ cells) + 200 mg/kg EADG	EAC (2×10 ⁶ cells) + 100 mg/kg salicin	EAC (2×10 ⁶ cells) + 200 mg/kg salicin	EAC (2×10 ⁶ cells) + 5-FU
Haemoglobin content (g/dl)	13.9 ^a	11.2 ^e	11.9 ^d	12.8 ^c	11.6 ^{de}	12.0 ^d	12.1 ^d	12.9 ^c	13.0 ^b
Total RBC*	6.1 ^a	4.3 ^{ef}	4.8 ^e	5.6 ^{bc}	4.5 ^f	5.1 ^c	5.0 ^d	5.7 ^{bc}	5.8 ^b
Total WBC**	8.1 ^f	14.8 ^a	11.8 ^b	10.3 ^c	9.4 ^d	8.6 ^{ef}	11.2 ^b	9.9 ^c	8.9 ^e
Lymphocyte (%)	65.8 ^a	23.9 ^f	57.2 ^d	62.9 ^b	55.1 ^e	60.8 ^c	58.8 ^b	63.4 ^c	65.0 ^a
Neutrophil (%)	32.0 ^f	73.9 ^a	39.7 ^c	35.2 ^d	40.8 ^b	36.4 ^{de}	38.5 ^c	33.7 ^d	32.9 ^{ef}
Monocyte (%)	3.3 ^c	2.1 ^f	2.9 ^d	3.8 ^b	2.7 ^e	3.4 ^c	3.1 ^d	3.9 ^b	4.1 ^a

* (cells/ml ×10⁹); ** (cells/ml ×10⁶)

Each point represents the mean (n = 10 mice per groups).

The values marked with the different letters show significant difference (Duncan's multiple range test, P < 0.05).

Table 3: Effect of different doses of methanol and ethyl acetate extract of the *Desmodium gangeticum* on different biochemical parameters in liver in EAC bearing mice.

Parameters	Normal Saline (0.5 ml/kg)	EAC control	100 mg/kg MEDG	200 mg/kg MEDG	100 mg/kg EADG	200 mg/kg EADG	100 mg/kg salicin	200mg/kg salicin	5-FU
Catalase*	2.62 ^a	1.58 ^{de}	1.91 ^d	2.41 ^{bc}	1.86 ^{de}	2.34 ^{bcd}	2.01 ^c	2.45 ^b	2.59 ^{ab}
SOD**	4.34 ^a	3.24 ^c	3.89 ^{bc}	4.25 ^{abc}	3.75 ^{bcd}	4.12 ^{abcde}	3.98 ^b	4.15 ^{abcd}	4.28 ^{ab}
GSH ⁺	2.39 ^a	1.72 ^{cd}	2.11 ^{bc}	2.22 ^{abcd}	1.98 ^c	2.19 ^{abcde}	2.15 ^b	2.24 ^{abc}	2.26 ^{ab}
Lipid peroxidation ⁺⁺	0.99 ^{cd}	1.48 ^a	1.21 ^{abcd}	1.08 ^{bc}	1.36 ^{ab}	1.26 ^{abc}	1.15 ^b	1.04 ^{bcd}	1.01 ^c

* Units / mg tissues; ** Units / mg Protein; + mg/g of tissue; ++ n moles MDA/ g of tissue

Each point represents the mean (n = 10 mice per groups).

The values marked with the different letters show significant difference (Duncan's multiple range test, P < 0.05).

DISCUSSION

The present study illustrated the effect of MEDG, EADG and salicin on EAC bearing mice, which significantly increased the life span of treated animal as compared to the EAC control. The reliable conditions for evaluating the value of any anticancer drug are prolongation of life span and decrease in the WBC count^[27]. The ascitic fluid is the direct nutritional source to tumor cells and the rapid increase in ascitic fluid with tumor growth could possibly be a means to meet more nutritional requirements of tumor cells.^[28] Furthermore the reduced volume of EAC and increased survival time of mice suggest the delaying impact of MEDG, EADG and salicin on cell division.^[29] The treatment with MEDG, EADG and salicin inhibited the tumor volume, viable cell count and enhanced survival time of EAC bearing mice. These finding suggest the anti-tumor effects of these extracts as well as salicin against EAC cell line.

Present study indicated that MEDG, EADG and salicin have significantly enhanced the erythrocyte count and hemoglobin level when compared to that of EAC control. The WBC level decreased when compared with the EAC control. These results indicate that MEDG, EADG and salicin possess little toxic effect on the hematological system.^[25,30] The tumors affect various functions of the vital organ, such as liver and kidney in human body or in experimental animals. Liver is most promptly affected even though when the site of the tumor does not interfere directly with organ function.^[31] It has been also demonstrated that tumor-bearing animals can experience a systemic change of enzymatic and non-enzymatic antioxidants in organs distinct from tumor sites.^[32-34] Taking these facts into consideration, the antioxidant related biomarker enzymes such as SOD and CAT and associated lipid peroxidation and GSH were estimated in the liver tissue of EAC and treated animal groups. In the present study, the EAC bearing mice showed significant deleterious effects on both free radicals scavenging systems like glutathione content and antioxidant enzymes such as SOD and CAT.

Lipid peroxidation has been associated in a number of deleterious effects such as increased membrane rigidity, osmotic fragility, decreased cellular deformation, reduced erythrocytes survival and membrane fluidity.^[35] Increase in the levels of TBARS indicates enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defence mechanism to prevent the formation of excess free radicals. Malonaldehyde (MDA) is the end product of lipid peroxidation was reported to be higher in cancer tissues than in non-diseased organ.^[36]

Excessive production of free radicals resulted in oxidative stress, which leads to damage of macromolecules such as lipids can induce lipid peroxidation in-vivo.^[37] In the present study indicates that the elevated levels of lipid peroxidation were observed in the liver of EAC bearing mice. However, the deleterious effects of reactive oxygen species are protected by MEDG, EADG and salicin administration. It may be due to the antioxidant properties of different active pharmacophore, which is responsible for the above said activity.

Reduced glutathione (GSH) is one of the most abundant tripeptide non-enzymatic biological antioxidants present in the liver. GSH is a potent inhibitor of neoplastic process, and it participates in endogenous antioxidant system that is found particularly in high concentration in liver and is known to have a key function in protective process.^[38] The present study indicates that the EAC control group produced elevation in the levels of lipid peroxidation and depletion in GSH content. With reference to this, the active role of GSH against cellular lipid peroxidation has been well recognized.

The SOD and CAT play an important role in the elimination of reactive oxygen species derived from the redox process of xenobiotics in the liver tissues. In correlation, it has been reported that EAC bearing mice showed decreased levels of SOD activity and this may be due to loss of Mn^{++} SOD activity in liver.^[39] Marklund et al.^[40] was observed inhibition of catalase activity in tumor cell lines, while Sun et al.^[39] reported diminished levels of SOD and CAT activity as a result of tumor growth. Similar findings were observed in the present investigation with EAC control mice. Thus elevation of lipid peroxidation is also known to be associated with cancer^[41] and decrease in SOD, GSH and CAT activities described in tumors is regarded as markers of malignant transformation.^[42] Therefore the significant elevation of GSH, SOD and CAT, and significant reduction in LPO by treatment confirms their antioxidant activity and free radical quenching property.

Preliminary phytochemical investigation indicated that the presence of flavonoid, alkaloids, tannins and terpenoids in MEDG and EADG and salicin is alcoholic β -glucoside^[43]. Flavonoids have been shown to possess antimutagenic and antimalignant effect^[44]. Furthermore, flavonoids have a chemopreventive role in cancer through their effect on signal transduction in cell proliferation and angiogenesis.^[44-46] The cytotoxicity and anticancer activity of MEDG, EADG and salicin are probably due to the presence of flavonoids. A number of scientific reports indicate certain terpenoids, steroids and phenolic compounds

such as tannins, coumarins and flavonoids to have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis.^[46,47]

Thus the present study suggests that the MEDG, EADG extracts and salicin isolated from *D. gangeticum* leaves possess potent anticancer activity and increase life span of the tumor bearing host

CONCLUSION

The pharmacological studies showed significant anti-cancer properties at the dose of 100 mg/kg and 200 mg/kg with methanol and ethylacetate extracts of leaves as well as salicin isolated from leaves of *D. gangeticum*. Thus, antitumor effect produced by the MEDG, EADG and salicin may be due to its flavonoids and glucoside compounds as well as its antioxidant potential. These extracts as well as salicin restored the mean survival time and decreased tumor volume count in treated mice. Thus MEDG, EADG and salicin appear to possess potent anticancer activity and thereby were able to increase the life span of EAC mice.

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