Cancer Chemother Pharmacol (1993) 33: 130–138

Cancer Chemotherapy and Pharmacology

Evaluation of the genotoxic, cytotoxic, and antitumor properties of *Commiphora molmol* using normal and Ehrlich ascites carcinoma cell-bearing Swiss albino mice

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Received: 15 March 1993/Accepted: 2 July 1993

Abstract. The genotoxic, cytotoxic and antitumor properties of *Commiphora molmol* (oleo gum resin) were studied in normal and Ehrlich ascites carcinoma cell-bearing mice. In normal mice, the genotoxic and cytotoxic activity was evaluated on the bases of the frequency of micronuclei and the ratio of polychromatic to normochromatic cells in bone marrow, which was substantiated by the biochemical changes in hepatic cells. The antitumor activity of *C. molmol* was evaluated from the total count and viability of Ehrlich ascites carcinoma cells and their nucleic acid, protein, malondialdehyde, and elemental concentrations in addition to observations on survival and the trend of changes in body weight. The tumors at the site of injection were evaluated for histopathological changes. Treatment with *C. molmol* (125–500 mg/kg) showed no clastogenicity but was found to be highly cytotoxic in normal mice. The results obtained in the Ehrlich ascites carcinoma cell-bearing mice revealed the cytotoxic and antitumor activity of *C. molmol* which was found to be equivalent to those of the standard cytotoxic drug cyclophosphamide. On the basis of the nonmutagenic, antioxidative, and cytotoxic potential of *C. molmol* as observed in the present study, its use in cancer therapy seems to be appropriate and further investigations are suggested.

Introduction

The oleo gum resin obtained from the stem of various species of *Commiphora* (Burceraceae) is traditionally used in incense and perfumes and for the treatment of different diseases [1]. *Commiphora* resin, locally called "mur" or "myrrh", is widely used by folk-medicine practitioners for the treatment of some inflammatory conditions as an antipyretic, an antiseptic, a stimulant, and a mouthwash [2, 3]. Recently, the antiinflammatory, antipyretic, and antihistaminic effects of *C. molmol* [4] and the antiarthritic potential of *C. mukul* [5] have been verified.

C. molmol has been demonstrated to contain volatile oils (up to 17%), resins (up to 40%), and gum (up to 60%). The volatile oils are composed of terpenes, sesquiterpenes, esters, cinnamaldehyde, cuminaldehyde, cumic alcohol, eugenol, heerabolene, lemonene, dipentene, pinene, *m*-cresol, and cadinene. The resins consist of α-, β-, and γ-commiphoric acids; commiphorinic acid; α- and β-heerabomyrrhols; heeraboresene; commiferin; campesterol; β-sitosterol; cholesterol; α-amyrone; and 3-epi-α-amyrin. On hydrolysis, the gum yields arabinose, galactose, xylose, and 4-*o*-methylglucuronic acid [2, 5–7]. Sesquiterpenes and their derivatives have been shown to be potent inhibitors of Walker-256 carcinosarcoma, P-388 lymphocytic leukemia, Lewis lung tumor, sarcoma 180, and cervical cancer [8, 9]. β-Sitosterol has been reported to decrease colon tumors in rats [10]. Eugenol has been found to be cytotoxic in isolated rat hepatocytes [11].

Many reports are available on the folklore importance of *Commiphora* species in the treatment of malignant tumors and cancer of the spleen, liver, stomach, breast, head, nose, and eye [12]. However, no scientific paper has been published that can support such effects for *C. molmol* resins. Therefore, the present study was designed to investigate the genotoxic, cytotoxic, and/or antitumor effects of *C. molmol* in view of its cytotoxic phytoconstituents and folklore importance.

Materials and methods

Plant material

Samples of oleo gum resin (*Commiphora molmol*) were obtained from different geographical locations and batches (India, 1984; Yemen, 1987; and Riyadh, 1992) and evaluated for their variability. Preliminary tests revealed a lack of differences in their cytotoxic potentials (Table 1). The oleo gum resin used in the present study was collected from a local market in Riyadh, Saudi Arabia.

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Table 1. Evaluation of the activity profile of different batches of *Commiphora molmol*

Treatment and dose (mg/kg daily)	Year of sample collection	Place of sample collection	Total number of cells counted ($\times 10^6$/ml, mean ± SE)	Percentage of viable cells (mean ± SE)
1. Negative control (D. W.)	–	–	–	–
2. Positive control (D. W.)	–	–	371.6±28.42	93.41±1.37
3. Cyclophosphamide (10)	–	–	190.0±14.22***	80.10±1.51***
4. *C. molmol* (500)	1984	India	260.0±7.68**	86.82±2.06*
5. *C. molmol* (500)	1987	Yemen	265.0±13.56**	84.74±1.30**
6. *C. molmol* (500)	1992	Riyadh	268.0±8.72**	83.72±2.01**

Groups 3, 4, 5, and 6 were statistically compared with group 2. Five mice were used in each group. D. W., Distilled water
*P <0.05, **P <0.01, ***P <0.001; Student's t-test

Animal stocks

Female Swiss albino mice (SWR) aged 5–6 weeks and weighing 20–25 g were obtained from the Experimental Animal Care Center, King Saud University, Saudi Arabia. The animals were fed on a Purina chow diet and water ad libitum and were maintained under standard conditions of humidity, temperature, and light (12 h, light/12 h dark cycle).

Dose and mode of administration

The highest dose of oleo gum resin used in the present study (500 mg/kg) had previously been reported to be pharmacologically active [4] and was found to be effective in a preliminary study on its cytotoxic activity. It was given orally to animals as a fresh aqueous suspension.

Studies on femoral and hepatic cells of normal mice

A total of 25 mice were randomly assigned to different control and treatment groups (5 mice in each group). The experimental groups of mice consisted of the following: group 1, control (distilled water); group 2, cyclophosphamide (CP, 100 mg/kg); group 3, *C. molmol* (125 mg/kg); group 4, *C. molmol* (250 mg/kg); and group 5, *C. molmol* (500 mg/kg). An aqueous solution of *C. molmol* was given orally (gavage) to groups 3, 4, and 5 for 7 days. CP was injected i. p. (group 2) 30 h before the animals were killed. In each case, animals were killed 30 h after the last treatment. The femurs were used for a micronucleus test, and the liver was excised and stored at –20° C until analyzed for nucleic acid and protein contents.

Micronucleus test. The micronucleus test procedure described by Schmid [13] was followed. The femoral cells were collected in fetal calf serum. After centrifugation, the cells were spread on slides and air-dried. Coded slides were fixed in methanol and stained in May-Gruenwald solution followed by Giemsa stain. The polychromatic erythrocytes (PCE/1000 per mouse) were screened for micronuclei, and reduction of the mitotic index was assessed on the basis of the ratio of polychromatic to normochromatic erythrocytes (PCE/NCE ratio).

Estimation of protein and nucleic acids. Total protein was determined by the method of Lowry et al. [14]. The method described by Bregman [15] was used to determine the levels of nucleic acids. Tissues were homogenized and the homogenate was suspended in ice-cold trichloroacetic acid (TCA). After centrifugation, the pellet was extracted with ethanol. The levels of DNA were determined by treating the nucleic acid extract with diphenylamine reagent and reading the intensity of the blue color at 600 nm. For quantification of RNA, the nucleic acid extract was treated with orcinol and the green color was read at 660 nm. Standard curves were used to determine the amounts of nucleic acids present.

Studies on Ehrlich ascites carcinoma cell-bearing mice

Ehrlich ascites carcinoma cells (EAC cells) supplied through the courtesy of Dr. C. Benckhuijsen, Amsterdam, Holland, were maintained by serial transplantations in female Swiss albino mice (SWR, bred at the Experimental Animal Care Center, King Saud University, Saudi Arabia) every 8 days. A total of 100 female mice were randomly allotted to different control and treatment groups (20 mice in each group). Ten mice in each group were used for evaluation of the parameters on cytotoxicity, biochemistry, and histopathology, and the remaining ten animals in each group were used for body-weight and survival studies. EAC cells (2.5×10^6 cells/mouse) were implanted (i. p.) into all experimental mice except those in the negative control group. The treatment was initiated 24 h after the tumor implantation and was continued for 10 days.

The experimental groups of mice consisted of the following: group 1, negative control (distilled water); group 2, positive control (distilled water); group 3, CP (10 mg/kg daily); group 4, *C. molmol* (125 mg/kg daily); group 5, *C. molmol* (250 mg/kg daily); and group 6, *C. molmol* (500 mg/kg daily). Aqueous solutions of CP and *C. molmol* were given orally (gavage). In each case, five animals were killed 24 h after the last treatment. Samples of peritoneal fluid were collected for analysis of cytotoxicity and viability and for storage at –20° C for further investigations. Tumor tissues obtained from the same animals were preserved for histopathological investigations. The group of animals meant for evaluations of body weight and survival were assessed for weight and mortality daily until their death.

Cytotoxicity and viability. The samples of peritoneal fluid were collected and studied for the viability and cytotoxicity of EAC cells with a hemocytometer using a dye-exclusion technique [16].

Biochemistry studies. The frozen peritoneal fluid samples were used for estimation of nucleic acids, protein, malondialdehyde, and different elements.

Determination of malondialdehyde concentrations. The method described by Fong et al. [17] was followed. The EAC cells were homoge-

Table 2. Effect of *C. molmol* treatment on the frequency of micronuclei in femoral cells of mice

Treatment and dose (mg/kg daily)	PCE screened	Percentage of micronucleated PCE (mean ± SE)	NCE screened	PCE/NCE ratio (mean ± SE)
1. Control (Distilled water)	5996	0.43±0.04	5690	1.10±0.09
2. Cyclophosphamide (100)	4472	4.20±0.23***	6700	0.67±0.10**
3. *C. molmol* (125)	5300	0.28±0.09	6500	0.81±0.10*
4. *C. molmol* (250)	5000	0.30±0.06	6600	0.76±0.12*
5. *C. molmol* (500)	5600	0.33±0.02	8000	0.72±0.08**

Groups 2, 3, 4, and 5 were statistically compared with group 1. Five mice were used in each group
*P <0.05, **P <0.01, ***P <0.001; Student's t-test

Table 3. Effect of *C. molmol* treatment on the protein and nucleic acid levels in hepatic cells of mice

Treatment and dose (mg/kg daily)	DNA (μg/100 mg tissue, mean ± SE)	RNA (μg/100 mg tissue, mean ± SE)	Total protein (mg/100 mg tissue, mean ± SE)
1. Control (Distilled water)	215.26±7.69	705.00±19.71	15.64±0.21
2. Cyclophosphamide (100)	174.82±2.45***	567.19±20.67***	13.67±0.31***
3. *C. molmol* (125)	206.72±8.48	646.90±14.68*	15.78±0.11
4. *C. molmol* (250)	196.34±5.92	617.36±11.06**	15.69±0.13
5. *C. molmol* (500)	191.53±2.45	567.19±20.67***	15.57±0.18

Groups 2, 3, 4, and 5 were statistically compared with group 1. Five mice were used in each group
*P <0.05, **P <0.01, ***P <0.001; Student's t-test

nized in TCA and the homogenate was suspended in thiobarbituric acid. After centrifugation, the optical density of the clear pink supernatant was read at 532 nm. Malondialdehyde bis(dimethyacetal) was used as the standard.

Elemental analysis. The EAC cells were prepared for elemental analysis following the method of Whitehouse et al. [18]. The peritoneal fluid was centrifuged at 3000 rpm for 5 min, and the pellet was digested in concentrated nitric acid and diluted for analysis of Na, K, and Ca. A Varian atomic absorption spectrophotometer (AA-775) was used for the analysis.

Histopathological procedure. The tumors that developed at the site of injection of EAC cells were excised and fixed in 10% formaldehyde. The preserved tumor tissue was processed for routine paraffin-block preparation using an American Optical rotary microtome. Sections with a thickness of about 5 μm were cut and stained with hematoxylin and eosin [19]. The slides were examined for histopathological changes such as alterations in the size of the tumor, in the proportional necrosis of tumor cells, and in the numbers of hair follicles by an observer who was blind with respect to the treatment groups.

Statistical analysis

The different parameters studied were subjected to statistical analysis with Student's t-test.

Results

Studies on femoral and hepatic cells of normal mice

Genotoxic effects. Commiphora molmol treatment (groups 3, 4, and 5) caused no significant difference in the incidence of micronucleated PCE in femoral cells of normal mice as compared with the controls (group 1). However, there was a statistically significant decrease in the PCE/NCE ratio in the treatment groups, indicating the cytotoxic potential of *C. molmol.* CP treatment (group 2) significantly increased the number of micronucleated PCE and decreased the PCE/NCE ratio. The cytotoxicity of *C. molmol* was comparable with that of cyclophosphamide (Table 2).

Biochemical effects. The levels of DNA and proteins in the hepatic cells were not affected by *C. molmol* treatment in the normal mice (groups 3, 4, and 5); however, there was a significant decrease in their RNA content as compared with the control value (group 1). CP treatment (group 2) significantly reduced the DNA, RNA, and protein contents of hepatic cells as compared with the control value. The decrease in RNA content induced by *C. molmol* was comparable with that caused by cyclophosphamide (Table 3).

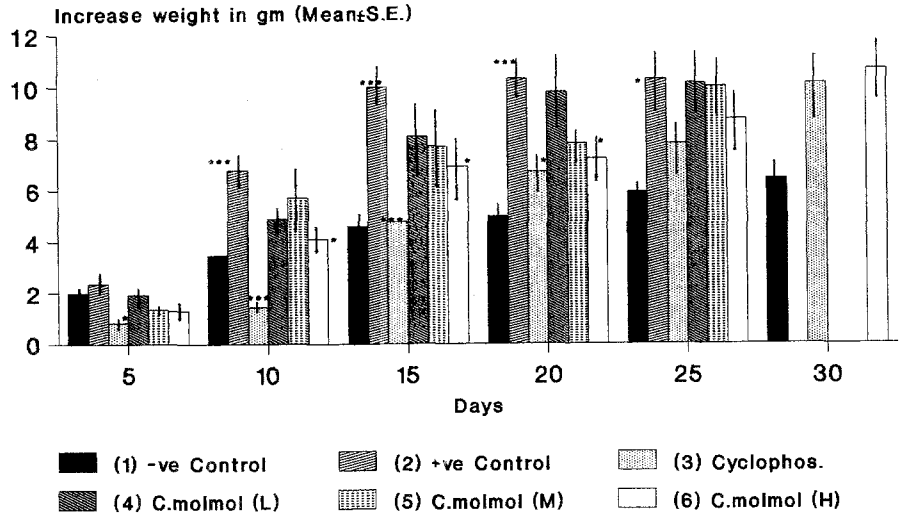

Fig. 1. Effect of *Commiphora molmol* treatment on body-weight changes of mice implanted with EAC cells. Ten mice were used in each group. ■, Group 1 (negative control); ▨, group 2 (positive control); ▩, group 3 (cyclophosphamide); ▧, group 4 (*C. molmol*, 125 mg/kg daily); ▦, group 5 (*C. molmol*, 250 mg/kg daily); □, group 6 (*C. molmol*, 500 mg/kg daily). Group 2 was statistically compared with group 1, and groups 3, 4, 5, and 6 were statistically compared with group 2. $^{*}P < 0.05$, $^{***}P < 0.001$; Student's *t*-test

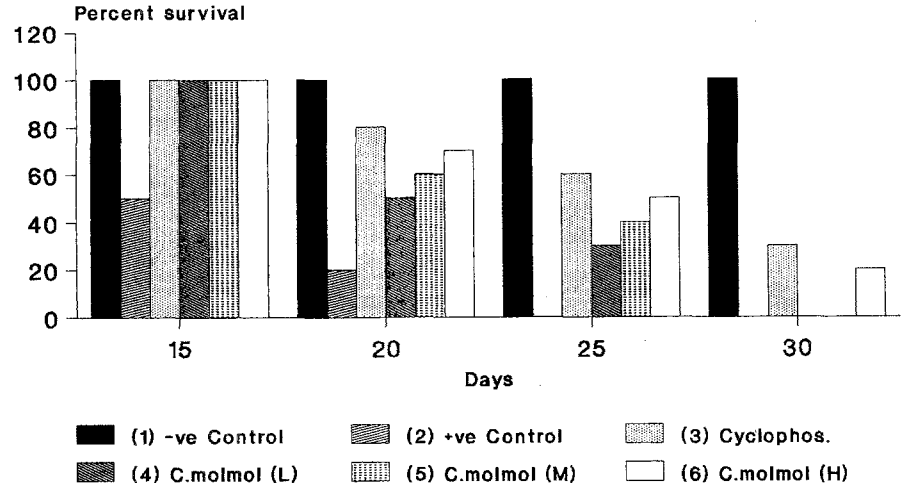

Fig. 2. Effect of *C. molmol* treatment on the percentage of survival (in days) of mice implanted with EAC cells. Ten mice were used in each group. ■, Group 1 (negative control); ▨, group 2 (positive control); ▩, group 3 (cyclophosphamide); ▧, group 4 (*C. molmol*, 125 mg/kg daily); ▦, group 5 (*C. molmol*, 250 mg/kg daily); □, group 6 (*C. molmol*, 500 mg/kg daily)

Studies on EAC cell-bearing mice

Effect on body weight. The EAC cell-bearing mice (group 2) showed a significant increase in body weight as compared with the normal mice in group 1. Treatment with *C. molmol* reduced the body weight of EAC cell-bearing mice, and this effect was statistically significant only at the highest dose (500 mg/kg daily) at 15 and 20 days after implantation. CP treatment also significantly reduced the body weight of EAC cell-bearing mice. The effect of *C. molmol* treatment (500 mg/kg daily) was comparable with that of cyclophosphamide (Fig. 1).

Effect on survival. The percentage of survival of EAC cell-bearing mice (group 2) was halved on the 15th day after implantation and no animal survived beyond the 21st day. The mean duration of survival in this group was 16.66 days. *C. molmol* treatment (500 mg/kg daily) increased the mean survival period to 25.1 days, with no animal living beyond day 31. The survival

Table 4. Effect of *C. molmol* treatment on the cytotoxicity and viability of EAC cells in mice

Treatment	Oral/gavage dose (mg/kg daily)	Total number of cells counted ($\times 10^6$/ml, mean ± SE)	Percentage of viable cells (mean ± SE)
1. Negative control	Distilled water	–	–
2. Positive control	Distilled water	375.00±25.37	91.17±1.20
3. Cyclophosphamide	10	181.00±8.17**	76.40±2.04**
4. *C. molmol*	125	306.00±24.48	90.98±1.42
5. *C. molmol*	250	299.00±11.84*	90.66±1.96
6. *C. molmol*	500	278.00±19.49*	85.71±1.72*

Groups 3, 4, 5, and 6 were statistically compared with group 2. Five mice were used in each group
$^{*}P < 0.05$, $^{**}P < 0.001$; Student's *t*-test

Table 5. Effect of *C. molmol* treatment on protein and nucleic acid levels in EAC cells of mice

Treatment and dose (mg/kg daily)	DNA (mg/100 ml P.F., mean ± SE)	RNA (mg/100 ml P.F., mean ± SE)	Total protein (mg/100 ml P.F., mean ± SE)
1. Negative control (Distilled water)	–	–	–
2. Positive control (Distilled water)	36.75±0.58	61.36±1.98	17.19±0.27
3. Cyclophosphamide (10)	23.64±1.34***	31.22±3.35***	14.53±0.56***
4. *C. molmol* (125)	36.31±1.27	60.00±2.03	16.94±0.20
5. *C. molmol* (250)	35.83±1.02	55.22±1.76*	16.65±0.16
6. *C. molmol* (500)	29.44±2.23*	46.81±3.76**	16.02±0.14**

Groups 3, 4, 5, and 6 were statistically compared with group 2. Five mice were used in each group. P.F., Peritoneal fluid
*P <0.05, **P <0.01, ***P <0.001; Student's t-test

Table 6. Effect of *C. molmol* treatment on the malondialdehyde concentrations in EAC cells of mice

Treatment	Oral/gavage dose (mg/kg daily)	Malondialdehyde levels (ng/ml P.F., mean ± SE)
1. Negative control	Distilled water	–
2. Positive control	Distilled water	358.00±14.19
3. Cyclophosphamide	10	336.67±8.81
4. *C. molmol*	125	344.00±11.11
5. *C. molmol*	250	319.00±8.86*
6. *C. molmol*	500	316.00±13.82*

Groups 3, 4, 5, and 6 were statistically compared with group 2. Five mice were used in each group. P.F., Peritoneal fluid
*P <0.05; Student's t-test

was 70% on the 20th day after implantation and 50% on the 25th day. The mean duration of survival in the lower dose range of *C. molmol* was 22.0 days at 125 mg/kg daily and 23.2 days at 250 mg/kg daily. An average of 50%–60% and 30%–40% animals were alive on the 20th and the 25th day after implantation, respectively. No animal survived beyond day 28. CP treatment (group 3) increased the survival period to up to 26.5 days, with 80% survival being observed on the 20th day after implantation and 60% survival being noted on the 25th day. No animal in this group lived beyond day 31. The percentage of survival and mean duration of survival of mice treated with *C. molmol* (500 mg/kg daily) were comparable with those of CP-treated animals (Fig. 2).

Effect of cytotoxicity and viability. C. molmol treatment (groups 4, 5, and 6) reduced the total number of peritoneal EAC cells. The decrease was statistically significant at the higher doses (250 and 500 mg/kg daily) as compared with the number of EAC cells obtained from mice in the positive control group (group 2). The viability of EAC cells was affected only at the highest dose of *C. molmol* (500 mg/kg

Table 7. Effect of *C. molmol* treatment on intracellular levels of some elements in EAC cells of mice

Treatment and dose (mg/kg daily)	Na (μg/ml P.F., mean ± SE)	K (μg/ml P.F., mean ± SE)	Ca (μg/ml P.F., mean ± SE)
1. Negative control (Distilled water)	–	–	–
2. Positive control (Distilled water)	607.00±42.03	439.20±23.20	32.80±2.40
3. Cyclophosphamide (10)	288.00±32.80***	272.00±47.20*	13.20±0.40***
4. *C. molmol* (125)	448.00±38.80*	398.40±56.40	20.47±1.75**
5. *C. molmol* (250)	410.40±47.60*	391.20±23.60	18.27±0.89***
6. *C. molmol* (500)	406.40±36.40**	365.60±24.00	16.78±0.76***

Groups 3, 4, 5, and 6 were statistically compared with group 2. Five mice were used in each group. P.F., Peritoneal fluid
*P <0.05, **P <0.01, ***P <0.001; Student's t-test

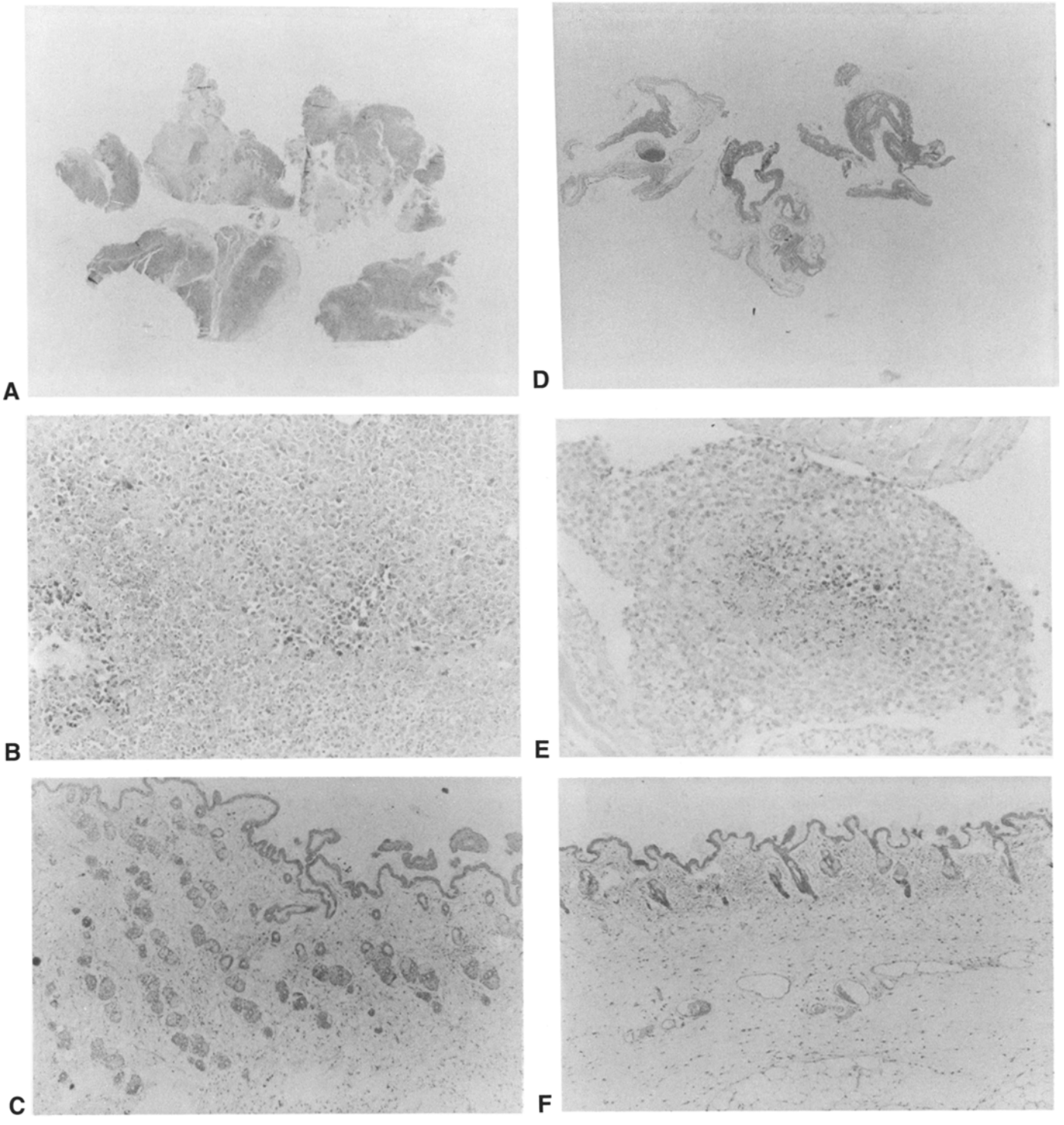

Fig. 3 (cont'd)

daily). CP treatment (group 3) also significantly affected the viability and reduced the total number of EAC cells. The cytotoxic activity of *C. molmol* was close to that of cyclophosphamide (Table 4).

Effect on the levels of nucleic acid and proteins. C. molmol treatment caused a dose-dependent reduction in the DNA, RNA, and protein contents of EAC cells (groups 4, 5, and 6) as compared with the positive control value (group 2). CP treatment (group 3) caused a highly significant reduction in the nucleic acid and protein contents as compared with the positive control value (group 2). The inhibition caused by *C. molmol* at the highest dose (500 mg/kg daily, group 5) was close to that induced by CP treatment (Table 5).

Effect of malondialdehyde concentration. The administration of *C. molmol* (groups 4, 5, and 6) resulted in a reduc-

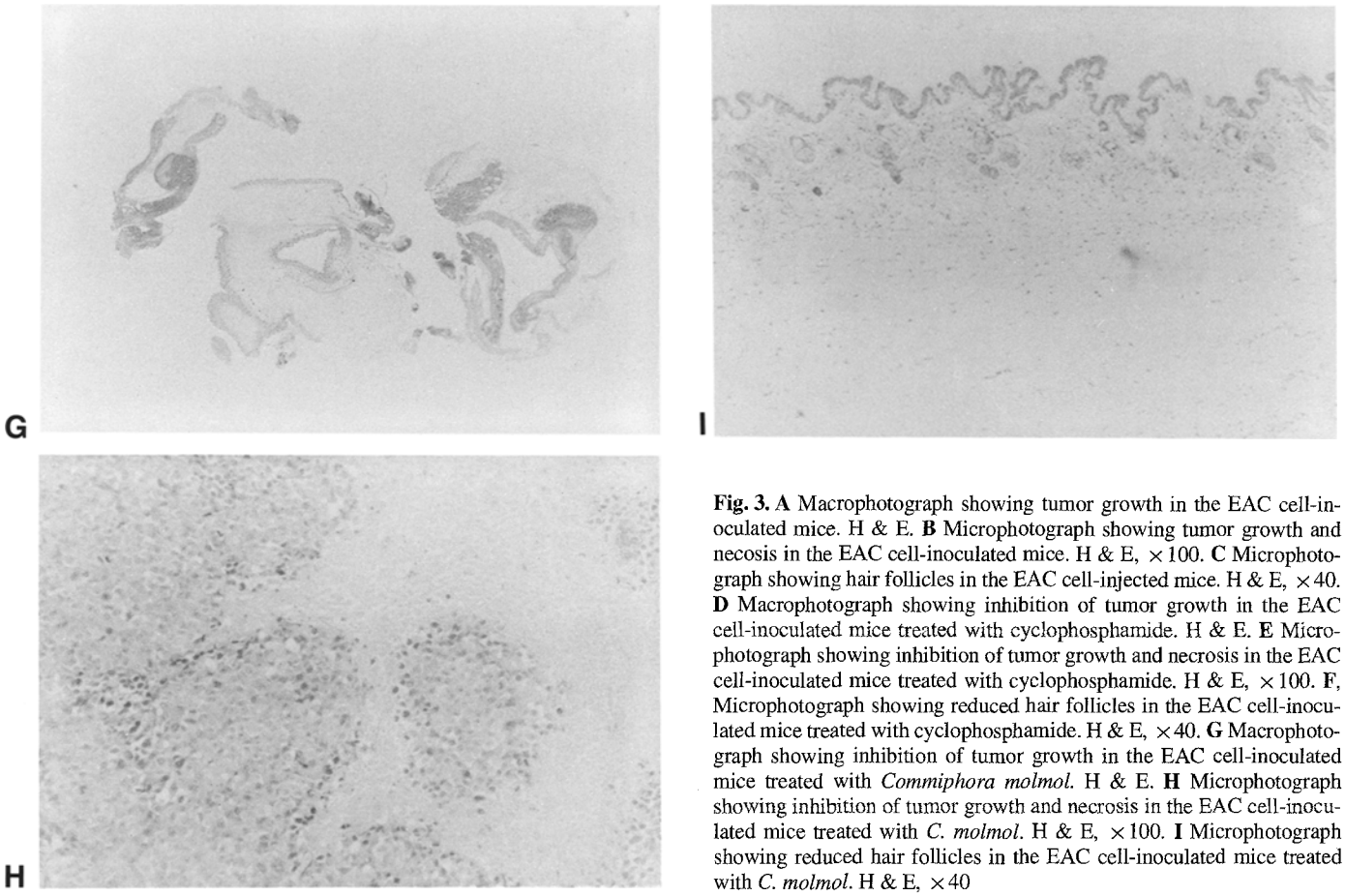

Fig. 3. A Macrophotograph showing tumor growth in the EAC cell-inoculated mice. H & E. **B** Microphotograph showing tumor growth and necosis in the EAC cell-inoculated mice. H & E, ×100. **C** Microphotograph showing hair follicles in the EAC cell-injected mice. H & E, ×40. **D** Macrophotograph showing inhibition of tumor growth in the EAC cell-inoculated mice treated with cyclophosphamide. H & E. **E** Microphotograph showing inhibition of tumor growth and necrosis in the EAC cell-inoculated mice treated with cyclophosphamide. H & E, ×100. **F**, Microphotograph showing reduced hair follicles in the EAC cell-inoculated mice treated with cyclophosphamide. H & E, ×40. **G** Macrophotograph showing inhibition of tumor growth in the EAC cell-inoculated mice treated with *Commiphora molmol*. H & E. **H** Microphotograph showing inhibition of tumor growth and necrosis in the EAC cell-inoculated mice treated with *C. molmol*. H & E, ×100. **I** Microphotograph showing reduced hair follicles in the EAC cell-inoculated mice treated with *C. molmol*. H & E, ×40

tion in malondialdehyde levels in the EAC cells of mice. The decrease was statistically significant at the higher doses (250 and 500 mg/kg daily) of *C. molmol* (groups 5 and 6) as compared with the value obtained in untreated EAC cell-bearing mice (group 2). CP treatment (group 3) failed to reduce significantly the malondialdehyde levels observed in EAC cells in the positive control group (group 2; Table 6).

Effect on Na, K, and Ca concentrations. Treatment with *C. molmol* decreased significantly the Na and Ca concentrations in EAC cells of mice (group 4, 5 and 6); however, the K levels were significantly reduced only at the highest dose (group 6). CP treatment (group 3) also significantly reduced the Na, K, and Ca contents of EAC cells as compared with the positive control value (group 2). The reduction induced by *C. molmol* in the elemental concentrations in EAC cells was found to be comparable with that caused by CP (Table 7).

Effect on histopathological changes. The tumors induced by EAC cells at the site of injection were very prominent and revealed a central area of necrosis surrounded by viable cells. The tumor was surrounded by edematous and mildly congested connective tissue and skeletal muscle fibers. There was no sign of inflammatory reaction surrounding the tumor (Fig. 3A–C). *C. molmol* treatment reduced the tumor size and increased the necrosis (Fig. 3H). Furthermore, the numbers of hair follicles in the overlying skin were lower in the treated animals (Fig. 3I) as compared with the positive controls (group 2; Fig. 3C). CP treatment similarly reduced the size of the tumor and increased the necrosis (Fig. 3E). The numbers of hair follicles were also reduced (Fig. 3F). The results of histopathological analysis revealed that *C. molmol* treatment caused changes that were similar to and comparable with those produced by CP (Fig. 3A–I).

Discussion

The results obtained in the present study revealed that *Commiphora molmol* significantly reduced the PCE/NCE ratio, indicating its cytotoxic potential. However, it was devoid of any clastogenic activity in the femoral cells of normal mice. CP treatment, on the other hand, was found to be highly clastogenic and showed significant cytotoxicity. The known cytotoxic potential of CP [20] was thus confirmed by this study, and the observed clastogenicity of CP may be attributed to its prooxidant properties [21, 22]. The cytotoxicity of *C. molmol* was comparable with that of CP.

No report is available in the literature on the clastogenicity and cytotoxicity of *C. molmol*; hence, a comparison is difficult. However, the present results concerning its cyto-

toxicity were supported by the reduction in the RNA content of hepatic cells observed in the same animals. Unlike treatment with CP, the treatment with *C. molmol* showed no significant effect on levels of DNA or protein. These findings indicate that the cytotoxic effect of *C. molmol* on hepatic cells is not due to intercalation with DNA. Although the phytoconstituents of *C. molmol* have not been investigated for their effects on the synthesis of nucleic acids, several natural drugs are known to inhibit RNA selectively [23, 24].

The results obtained in the studies on antitumor activity in EAC cell-bearing mice revealed that treatment with *C. molmol* decreased the body weight and increased the duration of survival of these animals. Although *C. molmol* treatment was not found to be toxic at the dose levels used in the present study, the late onset of mortality in this group appeared to be due to a delay in the proliferation of tumor cells. In a study on acute toxicity carried out in our laboratory, we found that *C. molmol* was nontoxic to mice in a dose range of 0.5–3.0 g/kg. Only at the highest dose were piloerection and hyperactivity observed (unpublished data).

The cytotoxic potential of *C. molmol* was clearly revealed by the finding that it significantly reduced the total number of EAC cells and affected their viability in the present study. These results are supported by the reduction caused by *C. molmol* treatment in DNA, RNA, and protein levels in EAC cells in the same animals. However, the observed inhibition of nucleic acids and proteins in EAC cells is not in agreement with our earlier observations in hepatic cells of normal mice, where *C. molmol* selectively affected the RNA content. The discrepancy between the results obtained in studies on hepatic cells and those on EAC cells may have been due to the sensitivity and rapid division of the latter cells. Previous reports have suggested that drug sensitivity may reflect differences in the intracellular concentration of enzymes and mediators of various target biochemical processes or repair mechanisms [25]. The observed cytotoxic potential of *C. molmol* may be attributable to its antitumor phytoconstituents, such as sesquiterpenes, steroids, and eugenol [2, 5–11]. CP treatment also inhibited the contents of nucleic acids and protein in EAC cells, which may be attributed to its prooxidant properties [21, 22].

Most cytotoxic drugs are known to increase the load of free radicals and stimulate lipid peroxidation in the body [26]. Malondialdehyde is a compound that increases oncogenic potentials [27] and, hence, any increase in the levels of malondialdehyde may cause the recurrence of cancer. In the present study, the malondialdehyde concentrations in EAC cells were significantly reduced after treatment with *C. molmol*. The reduced levels of malondialdehyde may be due to certain phytoconstituents of *C. molmol* such as cuminaldehyde, cinnamaldehyde, and eugenol, which possess good oxygen radical-scavenging and antimutagenic potential [28, 29]. The treatment with CP failed to reduce significantly the malondialdehyde concentrations in EAC cells, perhaps due to its oxidant property [21, 22]. Previous reports have suggested that CP increases lipid peroxide formation in the pulmonary, myocardial, hepatic, and renal cell membranes [21, 30, 31].

The results of our elemental analysis revealed that the Na, K, and Ca levels in EAC cells were reduced by treatment with *C. molmol*. Several studies correlate the intracellular content of Na and Ca to cellular proliferation and the inhibition of tumor growth [32–37]. The reduction in Na and Ca levels induced by *C. molmol* treatment substantiated the other results obtained in the present study, indicating the antitumor potential of *C. molmol*. Our results were further supported by histopathological investigations of the tumors, which clearly showed a reduction in tumor size, an increase in necrosis, and a decrease in the number of hair follicles after *C. molmol* treatment. These changes induced by *C. molmol* treatment were similar to those caused by CP treatment.

From our present investigation it appears probable that the cytotoxic and antitumor effects of *C. molmol* result from its ability to reduce levels of DNA, RNA, proteins, and Na. Although it is difficult at this stage to explain the exact mechanism of action of this drug, the cytotoxicity of *C. molmol* may be related to its phytoconstituents, such as sesquiterpenes, steroids, and eugenol [2, 5–11]. The present study (a) indicates that *C. molmol* is a potent natural cytotoxic and antioxidant drug that is nonmutagenic and (b) provides support to its folklore claims. Further studies are warranted to isolate and identify the cytotoxic constituents of *C. molmol* as well as their mode of action and to determine the safety of the oleo gum resin for medicinal use in cancer therapy.

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