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Antitumor Activity of *Urtica pilulifera* on Ehrlich Ascites Carcinoma in Mice

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Abstract: The anti-tumor activity of 20% methanol extract of *Urtica pilulifera* was evaluated against the Ehrlich Ascites Carcinoma tumor model in mice. The activity was assessed using hematological studies, solid tumor mass and survival time. Intraperitoneal injection of *Urtica pilulifera* crude extract with different doses increased the survival time. Hematological parameters and packed cell volume, which were altered by tumor inoculation, were restored. Solid tumor mass was also significantly reduced. The bioactive compounds which produced these activities were isolated and identified as flavonoids and phenolic acids.

Key words: Cancer, *urtica*, flavonoids, phenolics

INTRODUCTION

Much attention has been given to the primary prevention of cancer in daily life. Suppression of the tumor promotion step by the functional constituents of plant resources is expected to prevent cancer development. A number of products have been studied for anticancer activity on various models. This has resulted in the availability of more than 3000 effective anticancer drugs (Cuendet *et al.*, 2006).

Urticaceae family was reported as one of the effective medicinal plant. The main varieties identified under the *Urtica* species are *Urtica dioica* L., *U. urens* L., *U. pilulifera* L., *U. cannabina* L., *U. membranacea* Poiret, *U. kiovensis* Rogoff. These plants are being consumed without any report of adverse effect (Gunther, 1959; Ali-Shtayeh *et al.*, 2000). A less known *Urticaceae* member is *U. pilulifera* L. In Turkish traditional folk medicine, this plant is commonly used as a remedy for diabetes mellitus (Baytop, 1999). Kavalali *et al.* (2003) evaluated the pharmacological activity of using the seeds of this plant. They used the water extract of seeds by infusion method and investigated its hypoglycemic activity on streptozotocin (STZ) induced diabetic rats. Significant hypoglycemic effect was found at a dose of 100 mg kg⁻¹ after i.p. administration for 30 days. The antioxidant effect of two extracts (20% methanol and petroleum ether) of three part of *U. pilulifera* (herb, root and seeds) was studied (Mahmoud *et al.*, 2006). Their effect was dose dependant. Methanol extracts induced greater effect on the measured antioxidant parameters. Among all plant parts, herb methanol extract showed the best effect. Mahmoud (2006) determined the changes in proteins, total lipid and phospholipids in mice liver treated with *U. pilulifera* extracts. It was found that lipid and phospholipids (PL) concentrations were decreased. Petroleum ether extracts were more effective on lipid concentration while methanol extracts were more effective on PL concentration. On the other hand, the two extracts increased phosphatidylcholine, phosphatidylethanolamine, phosphatidic acid and phosphatidylinositol concentrations.

In general, different studies on the family *Urticaceae* showed a certain anti-tumor activity. The steroidal component of *Urtica dioica* roots extract reduced the tumor growth through inhibiting the

membrane Na⁺, K⁺ ATPase activity of the prostate (Hirano *et al.*, 1994) or modulation of sex hormone-binding globulin to its receptor on human prostatic membranes in case of prostate patients (Hryb *et al.*, 1995). The polysaccharides of 20% methanolic root extract reduced prostatic growth by 33.8% (Lichius *et al.*, 1999a). It also reduced proliferation of lymph node and epithelial carcinoma of prostate cells (LNCaP) (Lichius *et al.*, 1999b; Konrad *et al.*, 2000).

The polyunsaturated fatty acids separated from *Urtica dioica* were reported to inhibit 5- α -reductase which converts testosterone to dihydrotestosterone (Vahlensieck, 2002). *Urtica dioica* aqueous extract has another specific mechanism to treat prostate hyperplasia; it significantly inhibits adenosine deaminase (ADA) activity in prostate tissue in patients (Durak *et al.*, 2004). *Urticaceae* family has safe therapeutic option for benign prostate hyperplasia syndrome (BPS), especially for reducing irritative symptoms and BPS- associated complications due to the postulated antiphlogistic and antiproliferative effects (Schneider and Rubben, 2004). According to the previous studies on the plant extract, it was of interest to estimate its anti-tumor effect on Ehrlich ascites carcinoma mice and defined the best duration for treatment using herb methanol extract of *U. pilulifera*.

MATERIALS AND METHODS

Plant Material

Plants at 50% flowering of *Urtica pilulifera* were grown in a defined soil type and were air dried.

Preparative Extraction and Separation

Five hundred gram *Urtica pilulifera* powder were exhaustively extracted with aqueous methanol then filtered and concentrated under reduced pressure. A concentrated extract (70 mL) was extracted with chloroform then the protein was denaturated using ethanol at 20°C and centrifuged at 3500 rpm.

Fractionation of Crude Extract

The fractionation was carried out according to Starck *et al.* (1985).

Isolation and Purification of Flavonoids

The purification of major flavonoids was carried out according to Mabry *et al.* (1970).

Animals

Male albino mice (17-20 g) were procured from the animal house in National Research Center in Spring 2005. They were housed in microlon boxes in a control environment (temperature 30±2°C and 12 h dark/light cycle) with standard laboratory diet and water.

Cells

EAC cells were obtained from Cancer Research Center in Cairo. Intraperitoneal inoculation of 10⁶ cells/mouse was applied.

Determination of Anti-tumor Activity of *Urtica pilulifera*

The anti-tumor activity of *Urtica pilulifera* on Ehrlich Ascites Carcinoma in mice was evaluated according to Raj Kapoor *et al.* (2004).

The animals were classified into seven groups each of them contains fourteen mice: all of them were injected by 10⁶ cell of Ehrlich Ascites Carcinoma (EAC) except the negative control group. After 24 h of inoculation all animals were subjected to different treatments as follows:

Group I: Mice were injected with saline.

Group II: Mice inoculated with 10⁶ cell of Ehrlich Ascites Carcinoma (EAC) were injected with saline.

Group III: The mice were injected with flouro uracil (20 mg kg⁻¹ b. wt. /day).

Group IV: The mice were injected with 29 mg kg⁻¹ *Urtica pilulifera* aqueous methanol extract (20% MeOH) of herb as a single dose (0.25 mL mouse of 2000 ppm crude extract).

Group V: The mice were injected with 14.5 mg kg⁻¹ 20% MeOH crude extract for two dose intervals (0.25 mL mouse of 1000 ppm crude extract/five days).

Group VI: The mice were injected with 9.67 mg kg⁻¹ (0.25 mL mouse of 666 ppm/three days) at three doses intervals.

Group VII: The mice were injected with 2.9 mg kg⁻¹ (0.25mL mouse of 200 ppm/day) daily dose for ten days.

At the end of the experiment (after 10 days), six animals were used for collection of blood samples for measuring the hematological parameters. The other eight mice were left to determine the median survival time. In this period the animals were not given any treatment and were fed on standard diet to death.

Determination of Hematological Parameters

In order to detect the effect of *U.P. E* on the hematological status of EAC-bearing, mice a comparison was made among the different groups. After 24 h of inoculation, all mice were treated by applied doses as mentioned above. After ten days of treatment, blood samples were collected from eye blood vein using heparinized tubes and preserved in -80°C until used.

The white blood cell count, red blood cell count, hemoglobin, packed cell volume and differential white blood cell count were determined.

Determination of Hemoglobin in Mice Blood

Using cyanomethoglobin method (International Committee for Standardization in Hematology of the European Society, 1965), the intensity of the color is measured photometrically at 540 nm.

Calculation

$$\text{Hemoglobin concentration (g dL}^{-1}\text{)} = \frac{A_{540 \text{ nm}} \times 64458 \times 2.52}{44 \times 10^3 \times 10 \times 0.02}$$

Where:

Molecular weight of hemoglobin = 64458 mol.

Extinction coefficient of hemoglobin = 44×10³

Conversion factor from 1 L to 100 mL = 10

Total volume of cyanide reagent plus the blood sample = 0.02

Determination of Microhematocrite (Packed Cell Volume)

The determination of microhematocrite (Packed Cell Volume) was followed according to the method of Dacie and Lewis (1975).

Determination of Blood Cells Differentiation

The measurement of white blood cells count, red blood cells count and differentiated white blood cells was according to Docie (1958).

Determination of Tumor Solid

The tumor solid of treated mice, 5-flouro uracil treated mice and tumor bearing mice was estimated according to Kuttan *et al.* (1990).

The tumor mass was measured from the 11th day of tumor induction. The measurement was carried out every 5th days of a period of 30 days. The volume of tumor mass was calculated using the formula:

$$V = 4/3 \times \pi r^2$$

Where r is the mean of r^1 and r^2 which independent radii of the tumor mass.

Determination of Survival Time

Animals were inoculated with 1×10^6 cells/mouse on day 0 and treatment with UPE started 24 h after inoculated, at a doses mentioned before. The control group was treated with the same volume of 0.9% sodium chloride solution. All the treatments were given for ten days; the Median Survival Time (MST) of each group consisting of eight mice was noted.

The anti-tumor efficacy of UPE was compared with that of 5-fluoro uraical ($20 \text{ mg kg}^{-1} \text{ day}^{-1}$; intraperitoneally for 10days). The MST of the treated groups was compared with that of positive control group using the following equation:

$$\text{Increases of lifespan} = (T - C / C) \times 100$$

Where

T = No. of days treated animals survived

C = No. of days control animals survived

Then the obtained data were tabulated

RESULTS AND DISCUSSION

Interpretation of Compound 1

The ^1H - NMR analysis showed characteristic signals of quinic acid at 2.107, 2.2828, 5.36 and 5.3- 5.6 ppm as well as signals of caffeic acid from 6.1 to 7.6907 ppm. The ^1H - NMR spectrum (in CDCl_3) showed signals at 2.107 and 2.63 ppm for H- 6, signals at 2.2828ppm and at 3.1ppm for H- 2, signal at 5.36 ppm for H- 4 and signal at 5.6 and 5.32 ppm for H- 5 and H- 3, respectively. The above data were for quinic acid which in agreement with mass spectral fragmentation which gave base peak at m/z 324 and another characteristic peak at m/z 192 resulted from cleavage of quinic acid ring, mean while the caffeic acid signals were given at 6.65 ppm for H-7, 6.1 ppm for H- 8, 7.5272 ppm for H- 6, 7.5567 ppm for H- 2 and signal at 7.6907 ppm for H- 5. The UV spectral data showed two absorption bands at 277 and 347 nm which are typical for caffeoyl quinic derivatives.

The mass spectral data of compound 1 show base peak at M/z 324 resulted from cleavage of quinic ring to give ions at m/z 192 ($\text{C}_{10}\text{H}_8\text{O}_4$) and peak at M/z 324 ($\text{C}_{15}\text{H}_{16}\text{O}_8$). The peaks of main fragments at m/z 179 and at m/z 307 are resulted from removal of -OH group from each two ions. The above fragmentation pattern was in accordance with Scholz *et al.* (1993).

The IR spectrum reveals the presence of hydroxyl group at 3440.81 cm^{-1} , while bands at 2853.35 and 2956.9 cm^{-1} indicate the presence of -CH and - CH_2 groups and band at 1640 cm^{-1} for carbonyl group.

Interpretation of Compound 2

Compound 2 constituted 0.53% from aqueous methanolic extract of *Urtica pilulifera* herb. The ^1H - NMR spectrum (in CDCl_3), mass spectrum and UV absorption of compound 2 are typically dicaffeoyl malic derivative, according to Budzianowski (1990). The ^1H - NMR showed characteristic signals for malic acid at 2.25 and 3.6459 ppm also signals from 6.25 to 7.7358 ppm for caffeic acid, this is in agreement with mass spectral fragmentation which gave $[\text{M}]^+$ at m/z 460, main fragments at

m/z 389, 338 m/z and base peak at m/z 304. The UV spectral data showed three absorption bands at 277 nm (shoulder), 347 and 399 nm which are characteristics for dicaffeoyl malic acid.

The infrared spectrum revealed the presence of hydroxyl group at 3424.2 cm⁻¹, band at 1738.6 cm⁻¹ for carbonyl group and bands for -CH and -CH₂ groups were at 1460 and 1384.7 cm⁻¹, respectively as well as bands at 1596 cm⁻¹ for C = C also band at 2852.5 cm⁻¹ for -CH and -CH₂ groups.

The mass spectral data of compound 2 showed [M]⁺ at m/z 460 and then cleavage of caffeic acid's side chain gave ion fragments at m/z 391 and at m/z 71. The cleavage of caffeic acid's A- ring gave main fragment at m/z 339 and fragment at m/z 51, then loss of two hydroxyl groups from caffeic acid's B- ring gave main fragment of base peak at m/z 304. This is the first recorded isolation of caffeic acid derivatives from *Urtica pilulifera*.

The Main Flavonoids from Herb of *Urtica pilulifera*

Interpretation of Compound 3

Compound 3 constituted 1.179% from crude extract; the UV spectral data shown in the following Table indicate that the flavonoid nature is flavone apigenin glycoside which was glycosylated with rhamnose and xylose, this is in accordance with Mabry *et al.* (1970).

This expectation was supported by ¹H- NMR, IR and mass spectral data which showed that three characteristic signals of flavone aromatic ring at 6.2, 7.5, 7.685 and 8.52 ppm for H- 6, H- 5', H- 3' and H- 6', respectively and the carbonyl group of C- ring gave band at 1700 cm⁻¹, characteristic signal of rhamnose methyl group at 1.085 to 1.233 ppm which gave a sharp band at 1416.5 cm⁻¹ in IR, the protons of rhamnose were as follow; signal at 3.282 for H- 5'', 3.556 ppm for H- 2'', 3.826 ppm for H- 6'' and 4.6 ppm for H- 1'' and signals at 3.11 for H- 2 of xylose while sugar hydroxyl groups gave band at 3424.2 cm⁻¹ on IR. The UV spectral data and ¹H- NMR were according to Mabry *et al.* (1970).

The mass spectral data show [M]⁺ at m/z 548 and base peak at m/z 453 and main fragment at m/z 167 and m/z 149 which were produced from the cleavage of ring B to gave base peak at m/z 453 then cleavage of xylose moiety to give main fragment at m/z 167 while the cleavage of rhamnose moiety gave main fragment at m/z 167, this fragmentation pathway in accordance with that of Harborne *et al.* (1975). This is the first recorded occurrence of these flavonoids in *Urtica pilulifera*.

Interpretation of Compound 4

Compound 4 represented 1.911% from aqueous methanolic crude extract, UV spectral data of compound 4 present in Table 1 show one absorption band at 261 nm and one shoulder at 326 nm which means compound 4 is related to isoflavonoids group.

Compound 4 showed a bathochromic shift of band I with sodium methoxide treatment (from 326 to 351 nm (shoulder)) and with aluminum chloride to 378 nm with presence of shoulder at 288 by adding sodium acetate and then boric acid, this indicates the presence of free hydroxyl groups at C-5 and C-4' position and non free hydroxyl group at position C-7. Hydrolysis of compound 4 and comparison of its sugar moiety to authentic sugars showed two sugars; rhamnose and xylose. The

Table 1: The Ultra violet spectral properties of isolated flavonoids (compound 3 and 4)

Absorption	Flavonoid reagents						Color	
	MeOH	NaOMe	AlCl ₃	AlCl ₃ /HCl	NaOAc	NaOAc/ H ₃ BO ₃	UV	+NH ₄
Compound 3	268,333	244sh, 269,300sh, 384	244sh, 269,300s h, 384	273, 300sh, 344, 380	274, 300sh 338, 380	255sh, 268 353, 386	Purple	Yellow -green
Compound 4	261,326sh	270,305 sh, 350sh	271,306sh, 378	270,305sh, 378	261,288sh 325sh	261,288sh, 324sh	Purple	Purple

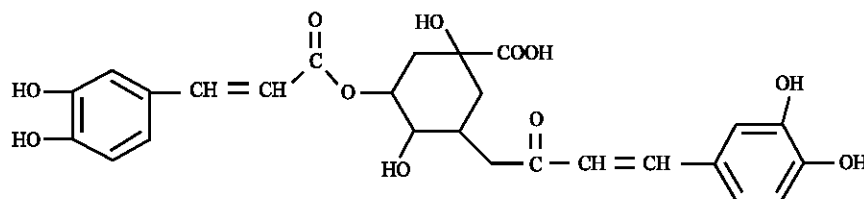
MeOH = Methanol, NaOAc = Sodium acetate, H₃BO₃ = Boric acid, NaOMe = Sodium methoxide, AlCl₃ = Aluminum chloride, HCl = Hydrochloric acid

above data were supported by ¹H- NMR spectral data, they showed signals at 8.513, 7.686, 7.485 and 6.177 ppm for H-6', H- 3', H- 5', H- 6 of aromatic ring but the characteristic signals of rhamnose methyl were at 1.097 to 1.285ppm and the other signals of rhamnose protons were at 3.507, 3.415, 3.22 ppm or H-6'', H-2'' and H-5'', signals of xylose protons were at 4.985 ppm, 4.145 ppm, 2.728 ppm for H-1'', H-6'' and H-2'', the ¹H- NMR data and UV spectral properties are in accordance with Mabry *et al.* (1970).

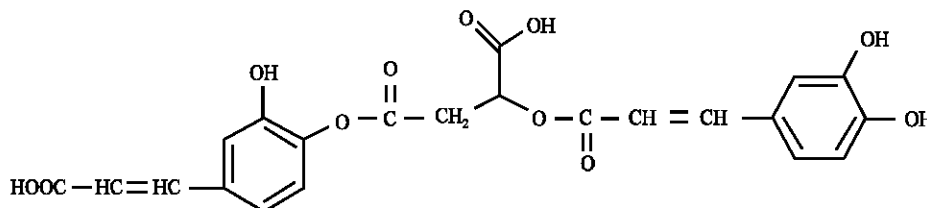
This compound has the same fragmentation pattern of compound 3 because both of them has the same structure but it differed in attachment of B- ring on C- ring which is at C-2 in compound 3 and at C-3 in compound 4, also they have the same sugars at position C- 7 on A- ring but the Mass spectral data showed characteristic fragmentation by cleavage of xylose to give main fragment at m/z 105, this is in agreement with Harborne *et al.* (1975).

The IR spectral analysis showed that compound 4 has a band of methyl group at 1414.3 cm⁻¹ on IR chromatogram, band at 3421.3 cm⁻¹ for hydroxyl group and band at 1700 cm⁻¹ for carbonyl group. This is the first recorded occurrence of flavonoids; apigenin and genstein glycoside in *Urtica pilulifera*.

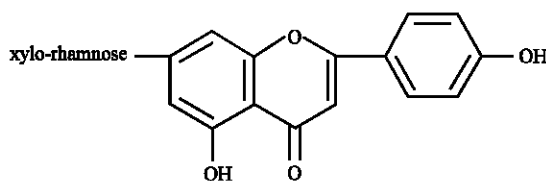
The structures of the isolated compounds are as follows:



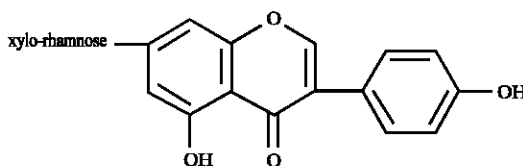
Compound 1: 3, 5-dicaffeoyl quinic acid



Compound 2: 2, 4-dicaffeoyl malic acid



Compound 3: Apigenin-7-rhamno-xylopyranoside



Compound 4: Genstein-7-rhamno-xylopyranoside

Table 2: Effect of 20% methanol extract of *U.P.* herb on hematological parameters of Ehrlich Ascites Carcinoma bearing mice

Groups	Hemoglobin (g %)	Packed cell volume (mm)	Red blood cells (\times million mm^{-3})	White blood cells (\times 1000 mm^{-3})	
(1) Control	11.18 \pm 0.83	33.25 \pm 2.57	5.29 \pm 0.53	11.85 \pm 0.59	
LSD	(2,4,6)	(2,4,6)	(2,4,6)	(2,5,6)	
(2) Tumor bearing mice	6.72 \pm 0.67	20.15 \pm 1.63	3.36 \pm 0.33	31.71 \pm 2.61	
LSD	(1,3,4,5,6)	(1,3,4,5,6)	(1,3,4,5,6)	(1,3,4,5,6)	
(3) One dose (2000 ppm)	9.05 \pm 0.01	27.66 \pm 0.19	4.53 \pm 0.003	12.86 \pm 0.33	
LSD	(2,4,6)	(2,4,6)	(2,4,6)	(1,2,3,4,6)	
(4) Two doses (1000 ppm)	10.49 \pm 0.53	31.47 \pm 1.6	5.24 \pm 0.27	17.94 \pm 3.28	
LSD	(1,2,3,5)	(1,2,3,5)	(1,2,3,5)	(2,5,6)	
(5) Three doses (666ppm)	10.22 \pm 0.72	30.64 \pm 2.17	5.11 \pm 0.36	12.87 \pm 0.80	
LSD	(2,4,6)	(2,4,6)	(2,4)	(2,5,6)	
(6) Daily doses (200 ppm)	9.22 \pm 0.84	27.66 \pm 2.53	4.61 \pm 0.42	21.4 \pm 0.99	
LSD	(1,2,3,5)	(1,2,3,5)	(1,2,3)	(1,2,3,4,5)	
ANOVA	F 22.39	F 22.57	F 16.53	F 70.73	
	P 000	P 000	P 000	P 000	
Deferential white blood cells (%)					
Groups	Basophile	Eosinophile	Neutrophile	Lymphocyte	Monocytes
(1) Control	3.75 \pm 0.5	2.25 \pm 0.5	28.75 \pm 1.5	60.25 \pm 2.23	5 \pm 0.00
LSD			(2,6)	(2,6)	(4)
(2) Tumor bearing mice	5 \pm 0.82	2.25 \pm 0.5	64 \pm 1.16	25.25 \pm 0.5	3.75 \pm 0.96
LSD		(5)	(1,3,4,5,6)	(1,3,4,5,6)	(4)
(3) One dose (2000 ppm)	4.75 \pm 2.87	2 \pm 0.00	29.25 \pm 2.63	59.75 \pm 2.06	4.25 \pm 2.36
LSD		(5)	(2,6)	(2,6)	(4)
(4) Two doses (1000 ppm)	2.75 \pm 0.96	2.25 \pm 1.26	27 \pm 1.41	62.0 \pm 1.63	6.0 \pm 0.82
LSD		(5)	(2,6)	(2,6)	(1, 2, 3, 5, 6)
(5) Three doses (666ppm)	3.5 \pm 0.58	3.5 \pm 0.58	27 \pm 1.16	61.5 \pm 0.58	4.5 \pm 0.58
LSD		(1,2,3,4,6)	(2,6)	(2,6)	(4)
(6) Daily doses (200 ppm)	4.5 \pm 1.92	2.25 \pm 0.96	43.25 \pm 2.36	44.0 \pm 4.12	6.0 \pm 2.83
LSD		(5)	(1,2,3,4,5)	(1,2,3,4,5)	(4)
ANOVA	F 1.25	F 2.1	F 283.47	F 81.47	F 5.73
	P ns	P ns	P 000	P 000	P 002

Effectiveness of any drug depends upon several parameters including drug concentration and duration of drug metabolism and /or secretion. Accordingly, total drug is given once or divided into specific doses. The present study shows that the most effective treatment regimes are to divide the total dose (29.5 mg kg^{-1}) into three equal doses over ten days. This dose appears to be sufficient to give its effect, before completely metabolized, a second dose is given. This effect probably because the extract concentration in serum is strongly associated with cancer cells (Zelenitsky *et al.*, 2002). Administrating the drug into singlet or divided into two doses is not effective as divided in three doses, which may be due to the fact that the drug given completely metabolized before its booster dose was given. On the other hand, dividing the dose into ten equal doses is not appropriate to affect the measured parameters.

Hematocrit level and total lymphocyte count had an impact on prognosis in univariate analysis. In tumor bearing mice red blood cell (RBC) count haemoglobin (Hb), packed cell volume (PCV) and lymphocytes were decreased while white blood cell (WBC) count (total and differential except lymphocytes) were increased (Table 2). Decrease in hemoglobin is common in cancer patients and, given its known adverse impact on physical functioning and quality-of-life variables including fatigue and cognitive function. Associations have been found between low Hb levels and decreased survival in cancer patients as well as solid tumors (Hasenclever and Diehl, 1998; Motzer *et al.*, 1999).

Present results were coincided with the fact that cancer has been well described for many years as a cause of microangiopathic hemolytic anemia and thrombocytopenia (Antman *et al.*, 1979). Recently, compelling evidence that immune responses are impaired in patients with oral squamous cell carcinoma (SCC) (Jiang *et al.*, 2007). Some studies have shown that a growing tumor burden correlates with aggravating changes in immunity. The leukocytes of the innate immune system, including

Table 3: Effect of *Urtica pilulifera* herb methanol extract on the survival of tumor bearing mice

Groups	Tumor bearing mice (untreated group)	Group treated with 5- flourouracil 1 20 mg kg ⁻¹ day ⁻¹	Treated groups			
			One dose	Two doses	Three doses	Daily doses
Tumor volume (cm ³) %	2.99	1.09 (63.55)	1.282 (57.12)	1.133 (62.11)	1.048 (64.95)	1.312 (56.12)
MST (day)	22	40	38	44	48	42
Increase in lifespan (%)	0.0	81.82	72.73	100	104.55	90.91

Each group contains 10 animals, Days of treatment = 10 days, MST: Median survival time, Lifespan= (T-C) Cx100 (T: survival days of treated mice, C: Survival days of tumor bearing mice)

neutrophils, macrophages and NK cells, infiltrated the tumor site for a multipronged killing response (Hicks *et al.*, 2006). The significant increase in WBCs and neutrophils in tumor bearing mice due to the fact that these cells are the first one to arrive at sites of infection, where they can release chemokines and proteases that can in turn recruit both nonspecific and specific immune effector cells (Coussens and Werb, 2002). They can also release toxic granules against neighboring cells, suggesting their potential anti-tumor activity (Di Carlo *et al.*, 2001). Blood analysis, in Table 2 shows that all hematological parameters of groups treated with *Urtica pilulifera* crude extract were improved significantly at different intervals. However, Group 6 in which the dose was divided into three parts showed the best improvement. The result were inparallel with Onyeyili *et al.* (2001) who found that administration of crude aqueous extract of *Nauclea latifolia* stem bark resulted in improved haemoglobin and leucocytosis values in worm-infected sheep due to flavonoid compounds.

The tumor mass volume (Table 3) was decreased due to the treatments with different levels. The experimental doses exhibited the best effect on life span percentage than 5-flourouracil which is reported as a drug for increment in life span. The increment percentages are 72.75, 100, 104.55 and 90.91% by treatments of whole, two, three and daily dose, respectively.

Administration of *Urtica P.* increases mean hemoglobin concentration and improves mice-reported outcomes. These results were in parallel with Lee *et al.* (2006) who found that hemoglobin reduces the cytotoxicity cancer and anticancer drugs, such as 5-FU and 5-DFUR, to colon cancer cells, which could be an adverse factor during chemotherapy in a clinical setting. It was also found that in male C57B1/6 mice with serum proteins characterized by a molecular weight of about 300 kDa. This complex can possess biological activity and contribute to the control of tumor growth (Sitdikova *et al.*, 2006).

The low effect of one dose may be due to that a potentiative dose could be needed; however this potentiative dose is preferably applied if the immune effect is the target one (Haque *et al.*, 2006). This finding corroborates the notion that the timing of administration may be crucial for affecting hematological parameter. At the same time, lower TMV was in daily dose. This was happened because the daily dose caused stress over mice as shown from WBCs and neutrophil results (Table 2) where the mice was already under stress from cancer itself.

The obtained results of *Urtica pilulifera* anti-tumor experiment indicate that 20% methanol extract possess ameliorative effect on hematological parameter in tumor cases. Meanwhile, significant restriction of growth Ehrlich's carcinoma was observed following prophylactic treatment on Swiss albino mice with neem (*Azadirachta indica*) leaf preparation (NLP-1 unit) once weekly for four weeks (Haque *et al.*, 2006) which may be due to the different extract constituent.

Meral and Kanter (2003) studied the effect of aqueous methanol extract of *Urtica dioica* on CCl₄ treated rats. Their results coincide with the present data. They found that *U. dioica*. extract improved red blood cells (non-oxygenated RBC's) count, packed cell volume, white blood cells count and hemoglobin percentage levels in case of diseases caused disturbances in hematological parameters.

The 20% aqueous methanol extract of *Urtica pilulifera* herb contains varied bioactive compounds as mentioned in phytochemical study (Table 1). Each of the above bioactive isolated compounds has an activity to suppress the tumor growth by different mechanisms and enhance the immune system for tumor suppression and/or as anti-tumor promotion or/and chemopreventive agent (Mansour *et al.*, 2006).

One of the causes of the tumor is the effect of free radicals (Hofseth and Wargovich, 2007). The present data coincides with the previous study in which *U. pilulifera* herb extract showed a good antioxidant activity. This support the anti-cancer effect of the plant extract. Also, Seeram *et al.* (2005) support our present studies. They found that the flavonoids, especially these contain C-7 and C-4' hydroxyl groups act as anti-mutagen and anti-malignant agent. The phytochemical studies showed that *U.P.* contains the same flavonoids. Another study was done by Gao *et al.* (2002) gives similar results. Zhou and Mi (2005) and Mak *et al.* (2006) explained the mode of action of genistein glycosides as anti-tumor drug.

U. pilulifera contains isoflavone genistein glycoside, tyrosine kinase inhibitor, inhibits proliferation of cancer cell through the induction of apoptosis as reported by Lunyin *et al.* (2003) with prostate cancer cell. They found that genistein glycoside suppressed the DNA binding activation of NF-kappa B and C/TBP beta and the production of lipopolysaccharide induced tumor necrosis factor-alpha (TNF a), interleukin (IL)1 B and IL-6 in both from the liver and sera. Another bioactive compound isolated from *U. pilulifera* herb extract was phenolic acid esters group. The extract contains two caffeic acid derivatives, 3, 5 dicaffeoyl quinic ester and dicaffeoyl malic ester; they significantly inhibit the cell growth and syntheses of RNA, DNA and protein in human colon adenocarcinoma cells as reported by Roa *et al.* (1992).

The ameliorative effect of *Urtica pilulifera* herb extract on growth Ehrlich's carcinoma may be due to the presence of bioactive compounds such as polysaccharide (Lichius *et al.*, 1999a) that reduce tumor cell proliferation by decreasing sialic acid amount and phospholipids in cancer cell membrane (Tong *et al.*, 1994; Xue *et al.*, 2003). In a previous study (Mahmoud, 2006), it was found that the plant extracts reduced the total phospholipids in mice liver. Accordingly, this may be one of the mode of action of the plant extract to act as anti-cancer.

In conclusion, the herb ME showed prophylactic effect in mice inoculated with EAC where it reduced the tumor risk factors in blood analysis. A high efficacy rate was that of 1/3 dose/three day. So we recommended using herb 20% methanol extract in large scale in future.

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