ORIGINAL RESEARCH

Anticancer activity of essential oils towards cancer cell lines and against EAC tumorinoculated mice

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ABSTRACT

Objective: The main objective of the study was to evaluate cytotoxic activity of essential oils using cancer cell lines and antitumor activity against Ehrlich Ascites Carcinoma transplanted Swiss albino mice.

Materials and Methods: Lemon oil and jasmine oil were tested for their *In vitro* cytotoxicactivity against the mouse fibroblast (L929) cell line and urinary bladder (RT4) cell lineusing MTT Assay as well as*In vivo*tumorstudies werecarried out to access the chemo preventive effect of lemon oil and jasmine oil through intraperitoneal transplantation of EAC in a healthy adult Swiss albino mice.

Results: Among the essential oils,lemon oil showed statisticallysignificant (*** $P< 0.0001$) cytotoxic activitytowards L929 cell lineRT4 cell line as compared to standard.Whereas jasmine oil exhibited statistically significant (*** $P < 0.0001$) cytotoxic activityonly towards RT4 cell line and were insignificant towards L929 cell line compared to standard.On EAC inoculated mice, lemon oil showed significant (**$P < 0.001$) reduction in the volume of tumor as well as in weight of tumor with increase in mean survival time and % ILS (**$P < 0.001$ and ***$P <0.0001$) compared to tumor control. Jasmine oil additionally reported slightreduction in tumor volume and tumor weight and were insignificant, but showed significant increase in hemoglobin count (*$P < 0.0001$), as well as in mean survival time and % ILS (**$P < 0.0001$) compared to tumor control.

Conclusions: Lemon oil has showed significant cytotoxic activity towards mouse fibroblast cell lineand urinary bladder cell line compared to jasmine oil and on EAC inoculated mice lemon oil at a dose of 400mg/kg showed significant reduction in the tumor volume as well as tumor weight and significant increase in hemoglobin count compared tumor control. Whereas jasmine oil showed significant cytotoxic activity only towards urinary bladder cell line and on EAC inoculated mice, jasmine oilat a dose of 400mg/kg significantly reduced the eosinophil count compared tumor control.Both the oils showed significant increase in MST and % ILS.

Key words: Anticancer activity, Essential oils, EAC tumor

INTRODUCTION

Cancer is an irregular proliferation of cells that starts with a gene mutation that alters cellular function, is triggered by several factors, and can be inherited or acquired.[1] These cells need metabolic reprogramming to start tumor formation and progression.Cancer is an important cause of death worldwide, accounting for almost 10 million deaths in 2020.[2] Chemotherapy is the most frequently used therapeutic option to treat cancer. However, it also destroys many healthy cells, and is hampered by the undesirable drug resistance effect.[3]There is a tremendous clinical need for the development of new anticancer drugs with low toxicity and high efficiency. Medicinal plants have become an interesting source of anticancer compounds because they are safe, have fewer side effects, and are easily accessible. Natural products play an essential role in the discovery and development of anticancer agents, especially plant-sourced anticancer drugs have made significant contributions to cancer treatment.[4] Essential oils are soluble in alcohol, ether, and fixed oils, but insoluble in water. These volatile oils are generally liquid and colourless at room temperature. They have a

characteristic odour, and usually liquid at room temperature. These volatile oils are widely used in the cosmetics industry, perfumery and also aromatherapy. [5] The anticancer effect of natural essential oils and their chemical components have been well documented and have been used in cancer prevention and treatment.[6]

Citrus Limon (L.)Burm.f. is a tree reaching 2.5-3 m in height with evergreen lanceolate leaves belonging to family *Rutaceae*.Flowers are white with a purple tinge at the edges of the petals. The fruit is an elongated, oval, pointed green berry that turns yellow during ripening. The most important group of bioactive compounds in*Citrus limon* are flavonoids such as hesperidin, hesperetin, naringin, apigenin, diosmin; quercetin and their derivatives.[7] Citrus fruits contain a range of key nutrients such as vitamin C, vitamin A, folate, potassium, selenium and dietary fiber. Citrus fruits, flowers and leaves are a store house of secondary metabolites like flavonoids (hesperidin, naringin, narirutin, nobiletin, tangeretin), essential oil (limonene, linalool), limonoids (limonin, nomilin), alkaloids (synephrine), carotenoids, coumarins, glucarates, anthocyanins and phenolic acids.[8]Lemon oil is used in the treatment of acute rheumatism and rheumatic gout, in some forms of acute tropical dysentery and diarrhoea.It is applied to check post partum haemorrhage and is highly prized medicine as a flavouring agent and also used as an antidote to some acro-narcotic poisons.[9]

Jasmine is derived from the Arabic word meaning "yasmin" or "gift from God." Jasmine oil (*Jasminumofficinale* L.) is commonly known as "mallige" belonging to family Oleaceae. It is popularly known as "Queen of the night" as well as "moonlight of the grove". There are more than 200 species of jasmine cultivated in the subtropics worldwide mainly for the fragrance for the perfume industry. The major countries involved in the production of jasmine absolute are Egypt and India and smaller producing countries include Morocco, Algeria, France, and Italy. Egypt produces approximately 70–80% of the world's jasmine absolute alone.The main constituents include methyl anthranilate, indole, linalool, ketone, benzyl acetate, cis-jasmone, and skatole. Jasmine oil is traditionally used in treatment of various diseases like arthritis, hepatitis, conjunctivitis, gastritis, and diarrhea.[10]

MATERIALS AND METHODS

DRUGS AND REAGENTS

Lemon oil (LO), jasmine oil (JO), 5- Flurouracil (5-FU), 3-(4,5–dimethyl thiazol–2–yl)–5–diphenyltetrazolium bromide (MTT),Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Minimal Essential Media (DMEM) and trypsin were purchased from Sigma- Aldrich (St.Louis, Mo, USA). We purchased glucose, ethylene diamine tetra acetic acid, antibiotics from Hi-Media Laboratories Ltd. (Mumbai, India). Dimethyl Sulfoxide (DMSO) and propanol were obtained from E.Merck Ltd., (Mumbai, India). Ehrlich Ascites Carcinoma (EAC) cell lines were procured from Amala Cancer Research Centre, Thrissur, Kerala, India

PRELIMINARY PHYTOCHEMICAL SCREENING

The essential oils were subjected for preliminary qualitative chemical analysis to reveal the presence of vital phytoconstituents.[11]The results are shown in (Table 1).

CELL LINES AND CULTURE MEDIUM

L929 (mouse fibroblast) cell line and RT4 (urinary bladder) cell line was procured from American Type Culture Collection (ATCC), India. Stock cells were cultured in DMEM and McCoy′s 5a medium with 2mM Glutamine respectively. Both the media were supplemented with 10% inactivated Fetal Bovine Serum (FBS), 1% penicillin (100 IU/ml) and streptomycin (100 µg/ml) in an humidified atmosphere of 5% CO_2 at 37ºC until confluent. The cells were dissociated using TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The viability of the cells are checked using trypan blue and centrifuged. Further, 50,000 cells / well of L929 and RT4 were seeded in a 96 well plate and it was incubated for 24 h at 37ºC, 5% CO_2 incubator.

ANIMALS

Adult Swiss Albino mice were used for studying theacute oral toxicity of test drugs. The mice weighing between 25-30g were procured from central animal house of H.K.E.S MotashreeTaradevi Institute of Pharmaceutical Sciences, Kalaburagi were used for above studies. The animals were housed in polypropylene cages and maintained under standard conditions (12 h light/dark cycle, 25±2^{0}C, 55% relative humidity) Animals were fed with standard pellet diet (M/s Champaka feeds & foods, Bangalore) with water *ad libitum.* Acute toxicity studies carried out in Research lab, Department of Pharmacology, H.K.E.'s MatoshreeTaradevi (HKECOP/IAEC/64/2014-19). *In vivo* liquid tumor model was designed, executed and monitored under in-house established protocols at Skanda Life Sciences Pvt Ltd, Bangalore

ACUTE ORAL TOXICITY

The acute oral toxicity tests were conducted in mice using lemon oil and jasmine oil. Acute oral toxicity tests were performed according to Organisation for Economic Co-operation and Development (OECD) guidelines 425.[12] Normal adult female Swiss albino mice weighing between 20 - 25 g were used for the study. The food, but not water, was withheld for 4 hbefore the extract was administered orally. The

essential oils were administered orally in doses of 2000 mg/kg, with three mice each dose. The mice were examined over 24 hours for behavioural, neurological, and autonomic characteristics, as well as any lethality or death over the next 48 hours.

PREPARATION OF TEST SOLUTIONS

For cytotoxicity studies, 10mg of test drug was separately dissolved in media containing 1% tween 80 and volume was made up with minimal essential medium supplemented with 2% inactivated FBS to obtain a stock solution with 1mg/ml concentration that has been filtered-sterilized. From this, serial two fold dilutions were made for cytotoxic testing.

***IN VITRO* CYTOTOXIC ACTIVITY**

PRINCIPLE: The ability of the cells to survive a toxic assault has been the basis of most cytotoxicity assays.[13]This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay is dependent on the quantity of cells present as well as the level of mitochondrial activity in each cell. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used.

PROCEDURE: Test samples were placed in each well of the 96 well microtiter culture plate. The L929 monolayer cells were subjected for trypsinization and the cell count was adjusted to 5.0 x 10^5 cells/ml using DMEM containing 10% FBS. The RT4 monolayer cells were subjected to trypsinization and the cell count was adjusted to 5.0 x 10^5 cells/ml using McCoy′s 5a containing 10% FBS. To each well of the 96 well microtiter plate, 100 µl of the diluted cell suspension (50,000 cells/well) was seeded on each scaffold and cells seeded on cell culture plate. The plates were then incubated at 37ºC for 1 day in 5% CO_2 atmosphere. After 24 h, the test solutions in the wells were discarded and 100 µl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were gently shaken and incubated for 4 h at 37ºC in 5% CO_2 atmosphere. The mitochondrial dehydrogenase enzymes of viable cells cleave the tetrazolium ring to an insoluble purple formazan. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the intracellular formed formazan and the absorbance was measured using a microplate reader at a wavelength of 590 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves for each cell line.

CALCULATING PERCENTAGE GROWTH INHIBITION

$$\% \text{ inhibition} = 100 - \left(\frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \right) X\ 100$$

OD = Optical Density

***IN VIVO* ANTICANCER ACTIVITY**

STUDY DESIGN

The EAC cells (1x10^6cells/mouse) were maintained in male Swiss albino mice by intraperitoneal transplantation under standard laboratory conditions throughout the study. The entire group animals were injected with EAC cells except for the normal group. The mice were divided into five groups comprising six animals (n=6) in each group. Grouping of animals was done on the last day of the acclimatization period by body weight stratification method.

TREATMENT GROUPS (N=6)

Group 0	Normal (sham)
Group I	EAC control
Group II	Induced EAC cells + 5- flurouracil (20mg/kg) i.p
Group III	Induced EAC cells + Lemon oil(400mg/kg) i.p
Group IV	Induced EAC cells + Jasmine oil (400mg/kg) i.p

TUMOR INDUCTION

Group 0 is considered as normal control and was administered with suitable vehicle. Group I, II, III, IV were inoculated with 1x10^6EAC cells intraperitoneally and this considered as day ‘0’. Group I will serve as EAC control. Treatment was started after 24 h of tumor induction where Group II animals were injected with standard drug (5-FU) with a dose of 20mg/kg from 1st day to 15th day. Group III and IV animals were treated with test drugs of lemon oil and jasmine oil at a dose of 400mg/kg respectively started after 24h of tumor induction from 1st day to 15th day. Body weight was taken from day 1 to day 15. On day 15, blood collection from retro-orbital plexus was carried out and the samples (0.1ml) in EDTA were used for assessment of hematological parameters such as hemoglobin (Hb) content, red blood cell (RBC) count, total white blood cell (WBC) count, eosinophils, monocytes, neutrophils, lymphocytes (%) and total serum protein was measured. On the same day total tumor fluid was withdrawn from the animals and tumor cell parameters such as total cells, viable and non-viable count were done using Neubauer chamber under the light microscope. Hematological

parameters were carried out using kit based assay and protein estimation was done using Bradford method.

RESULTS

PRELIMINARYPHYTOCHEMICALSCREENING

Phytochemical analysis was carried out to confirm the presence of phytocontituents in the extracted oils and it has shown the presence of carbohydrates, flavonoids, steroids and terpenoids, tannins in lemon oil and presence of carbohydrates, alkaloids, flavonoids, steroids and terpenoids, glycosides in jasmine oil. The results are shown in (Table 1)

ACUTE TOXICITY STUDIES

In acute oral toxicity studies, no changes in the behavior and autonomic profiles as well no mortality were observed in all mice up to the dose of 2000 mg/kg with lemon oil and jasmine oil.

CYTOTOXIC ACTIVITY TOWARDS CANCER CELLS

Lemon oil and jasmine oil were evaluated against mouse fibroblast and urinary bladder cell line. The cancer cell lines namely L929 and RT4 were exposed to increasing concentrations of essential oils. Cell viability was determined using MTT assay. With reference to (Table No.2) lemon oil and jasmine oil showed different cytotoxic activities towards these cancer cell lines. The results revealed that lemon oil exhibited strongest cytotoxicity towards L929 cell line with IC_{50} value of 433.7µg/ml. At a concentration of 1000 µg/ml, 500 µg/ml, 250 µg/ml & 125µg/ml lemon oil has showed statistically significant (***P< 0.0001) anticancer activity towards L929 cell line, whereas jasmine oil at different concentrations didn't show any significant anticancer activity towards L929 cell line with IC_{50} value of 950.2µg/ml compared to standard. Here the both essential oils were insignificant at a low concentration of 31.25 µg/ml. Towards RT4 cell line, lemon oil at a concentration of 1000 µg/ml, showed statistically significant (***P< 0.0001) anticancer activity when compared to standard with IC_{50} value of 671.8 µg/ml. Whereas at 500 µg/ml, 250 µg/ml and 125µg/ml concentration jasmine oil showed statistically significant (**P< 0.001) anticancer activity towards RT4 cell line when compared to standard with IC_{50} value of 1433 µg/ml. The results are shown in Table-2 & Table-3

IN VIVO ANTICANCER ACTIVITY

TUMOR PARAMETERS

BODY WEIGHT

Tumor bearing mice showed a significant (*P< 0.01) increase in body weight (34.83±1.62) as compared to normal group (24.83±0.47). The standard 5-Flurouracil (20mg/kg) treatment group showed significant (*P< 0.01) reduction in weight gain (27.33±1.58) compared to tumor control. Animals treated with lemon oil at a dose of 400mg/kg significantly reduced (*P < 0.01) the body weight (27.83±1.4) as compared to tumor control. Jasmine oil at a dose of 400mg/kg did not show any significant reduction in body weight as compared to tumor control.

TUMOR VOLUME AND TUMOR WEIGHT

The standard 5- Flurouracil (20mg/kg) showed significant (*p< 0.01) reduction in tumor volume (5.66±0.76) compared to tumor control, while animals treated with lemon oil at a dose of 400mg/kg significantly reduced (**P < 0.001) the tumor volume (3.75±1.4) compared to tumor control (13.66±3.37). Jasmine oil also showed reduction in tumor volume (9.83±1.7) at a dose of 400mg/kg but were not significant as compared to tumor control. Reduction in the tumor weight was also observed with lemon oil at the same above dose, where they significantly reduced (**P <0.001) the tumor weight (4.00±0.32) compared to EAC control (10.16±0.40). Significant reduction was not much seen with standard drug (5-FU) and jasmine oil compared to tumor control. The results are displayed in (Table 3).

MEAN SURVIVAL TIME AND % INCREASE IN LIFE SPAN

Lemon oil and jasmine oil at a dose of 400mg/kg significantly increased (**P < 0.001 and ***P < 0.0001) mean survival time (14.50±5.00 &15.00±5.00) compared to tumor control (7.33±2.45). Standard drug (5-FU) also significantly increased (**P< 0.001) the mean survival time (13.66±0.88) compared to control. The above oils at same dose also significantly increased the life span of all group animals including standard group compared to tumor control animals. Lemon oil and jasmine oil were more effective in increasing the life span of animals compared to standard group.

VIABLE, NON-VIABLE AND TOTAL CELLS

Animals treated with lemon oil and jasmine oil at a dose of 400mg/kg did not show any significant results in the viable, non –viable & total cell count compared to EAC control.

HEMATOLOGICAL PARAMETERS

HEMOGLOBIN COUNT

Tumor control group animals showed reduction in the hemoglobin levels as compared to normal group animals. Standard drug (5-FU) slightly increased the hemoglobin level compared to tumor control. Jasmine oila dose of 400mg/kgshowed significant increase (*P< 0.01) in hemoglobin level as compared to tumor control. Lemon oil a dose of 400mg/kg also showed increase in hemoglobin level but were insignificant. The results are displayed in (Table 4).

RBC AND WBC COUNT

Tumor control group animals showed reduction in the RBC levels as compared to normal group animals.

Animals treated with lemon oil and jasmine oil at a dose of 400mg/kg showed slight increase in RBC count (9.75±0.49 & 9.87±0.91) but were not significant compared to tumor control (8.02±0.50). Standard drug (5-FU) slightly increased the RBC count when compared to control. WBC counts were increased in tumor control (37300.0±5898.1) compared to normal (2816.6±307.0). Lemon oil at a dose of 400mg/kg significantly decreased (***P< 0.0001) WBC count whereas the activity of jasmine oil at same dose were insignificant as compared to control.

DIFFERENTIAL COUNT OF WBC

Tumor control group animals showed increase in neutrophil count (56.17±3.65) compared to normal (22.83±1.30). Standard drug (5-FU) showed significant reduction (*P <0.01) in neutrophil count (40.17±2.64) and even lemon oil showed significant reduction (***P < 0.0001) in neutrophil count (25.67±1.65) as compared to control. Here animals also showed increase in eosinophil count (9.00±0.52) compared to normal (1.83±0.40).Lemon oil and jasmine oil at a dose of 400mg/kg significantly reduced (**P < 0.001) the eosinophil count (5.17±0.87 & 5.17±0.75) compared to control. Whereas in case of monocyte and lymphocyte count significant changes were not observed with both the essential oils compared to control.

BIOCHEMICAL PARAMETER

TOTAL SERUM PROTEIN

Tumor control group animals showed increase in serum protein (26.65±1.35) compared to normal (8.13±0.78). Standard drug (5-FU) showed significant decrease (***P< 0.0001) in serum protein (14.88±0.41) compared to tumor control. Lemon oil and jasmine oil also increased the level of serum protein but were not significant as compared to control. The results are displayed in (Table 4).

HISTOPATHOLOGICAL STUDIES

The histopathological observation of liver sections of normal, EAC tumor control, standardand test drug treated animals were collected at the end of experimental periods. In normal untreated animals no histological changes were observed. EAC induced animals showed central vein dilatation, van-Kuffer cells and vacuolated Hepatocytes. Animals treated with standard drug (5-FU) showed vacuolated nuclei of hepatocytes and some animals show dilatation of central and portal vein. Prominent central vein dilatation and vacuolated nuclei of hepatocytes were observed in all animals treated with lemon oil and jasmine oil.

Table 1: Preliminary phytochemical screening of essential oils

Sl. No.	Phytoconstituents	Chemical tests	Lemon oil	Jasmine oil
1	Carbohydrates	Molisch test	+	+
		Fehlings test	-	+
2	Alkaloids	Dragendorff's test	-	+
		Hagers test	-	+
		Wagners test	-	+
3	Flavanoids	Shinoda test	+	+
4	Steroids and Terpenoids	Salkowaski test	+	+
		Libermann-Burchard test	+	+
5	Glycosides	General test for glycosides	-	+
6	Tannins	Ferric chloride test	+	-
7	Proteins and Amino acids	Biuret test	-	-
		Ninhydrin test	-	-
8	Saponins	Foam test	-	-

+ = Present-= Absent

Table 2: Cytotoxic properties of test drugs against L929 cell line

Sl. No	Name of Test Compound	Test Conc (µg/ml)	% Cytotoxicity	IC_{50} (µg/ml)
1.	Standard 5-Fluorouracil	1000	57.61±1.8	572.2 µg/ml
		500	39.49±0.8	
		250	31.12±1.4	
		125	22.83±1.7	
		62.5	11.95±0.5	
		31.25	9.24±0.7	
5.	Lemon oil	1000	94.95±0.1***	433.7 µg/ml
		500	75.32±0.5***	
		250	51.69±1.0***	
		125	42.09±1.8***	
		62.5	29.55±1.6	

		31.25	16.90±2.0	
6.	Jasmine oil	1000	60.32±1.6	950.2µg/ml
		500	39.97±1.6	
		250	28.14±0.9	
		125	19.62±1.8	
		62.5	11.89±2.0	
		31.25	7.47±2.1	

Values are Mean ± S.E.M., and statistical analysis by one-way ANOVA followed by ***Dunnett's*** test using GraphpadInstat software.
* indicates $p< 0.01$, ** indicates $p< 0.001$, *** indicates $p< 0.0001$ compared 5-flurouracil.
The above results have shown ***$p< 0.0001$ compared with control.

Table 3: Cytotoxic properties of test drugs against RT4 cell line

Sl. No.	Name of Test Compoun	Test Conc (µg/ml)	% Cytotoxicity	IC_{50} (µg/ml
1.	Standard 5-Fluorouracil	1000	63.24±1.3	226.3 µg/ml
		500	55.07±2.5	
		250	40.15±0.9	
		125	29.00±1.6	
		62.5	19.00±1.2	
		31.25	10.35±0.6	
2.	Lemon oil	1000	76.10±0.9***	671.8 µg/ml
		500	47.94±2.7	
		250	36.47±2.5	
		125	25.09±2.5	
		62.5	12.52±2.2	
		31.25	4.20±0.9	
3.	Jasmine oil	1000	56.40±1.3	1433 µg/ml
		500	35.73±0.8***	
		250	21.22±1.3***	
		125	12.08±0.9***	
		62.5	9.17±1.2	
		31.25	2.70±1.2	

Values are Mean ± S.E.M., and statistical analysis by one-way ANOVA followed by **Dunnett's**test using GraphpadInstat software.
* indicates $p< 0.01$, ** indicates $p< 0.001$, *** indicates $p< 0.0001$ compared 5-flurouracil. The above results have shown ***$p< 0.0001$ compared with control

Table 4: The effect of lemon oil and jasmine oil on tumor parameters like body weight, tumor volume, tumor weight, mean survival time (MST), percentage increase life span (%ILS), viable,non-viable and total cell count in EAC bearing mice.

Parameter	Normal (Negative control)	EAC Control (Positive control)	5-FU (20mg/kg)	LO (400mg/kg)	JO (400mg/kg)
Body weight (g)	24.83±0.47	34.83±1.62	27.33±1.58*	27.83±1.4*	33.00±2.1
Tumor volume (ml)	-	13.66±3.37	5.66±0.76*	3.75±1.4**	9.83±1.7
Tumor weight (g)	-	10.16±0.40	9.83±0.47	4.00±0.32**	9.16±0.40
MST (days)	15.00±0.00	7.33±2.45	13.66±0.88**	14.50±5.00**	15.00±5.00***
% ILS	-	0.00	47.06**	76.47***	76.47***
Viable cells ($x10^6$ cells/ml)	-	1407.0±292.4	643.1±257.5	620.5±209.2	1622.3±277.8
Non-Viable cells ($x10^6$ cells/ml)	-	358.8±230.0	529.6±129.8	180.0±37.5	232.3±105.6
Total cells	-	1765.8±489.7	1794.3±528.0	817.1±246.2	1778.6±385.6

All values are expressed as mean ± S.E.M (n = 6 mice per group) and statistical analysis was carried out using One-way ANOVA followed by Dunnett's test of significance.
***P*< 0.01, when treatment group is compared with control
** *P*< 0.001, when treatment group is compared with control
****P*< 0.0001, when treatment group is compared with control

Table 5: The effect of lemon oil and jasmine oil on hematological and biochemical parameters like Hemoglobin content, RBC count, WBC count, Neutrophil count, Eosinophil count, Monocyte count, Lymphocyte count and Total serum protein.

Parameter	Normal (Negative control)	EAC Control (Positive control)	5-FU (20mg/kg)	LO (400mg/kg)	JO (400mg/kg)
Hb (g%)	9.43±0.20	6.10±0.34	6.90±0.28	7.38±0.39	7.55±0.57*
RBC (10^6/cu mm)	10.02±0.56	8.02±0.50	8.27±0.57	9.75±0.49	9.87±0.91
WBC (10^3cells/cu mm)	2816.6±307.0	37300.0±5898.1	26733.3±1370.8	15715.0±2712.4***	29900.0±3830.2
Neutrophils (%)	22.83±1.30	56.17±3.65	40.17±2.64*	25.67±1.65***	44.67±6.66
Eosinophils (%)	1.83±0.40	9.00±0.52	6.33±1.09	5.17±0.87**	5.17±0.75**
Monocytes (%)	1.50±0.22	1.50±0.22	1.50±0.22	1.83±0.17	1.50±0.22
Lymphocytes (%)	28.50±3.01	48.33±2.55	42.67±1.67	57.33±2.70	54.67±2.96
Total serum protein (mg/ml)	8.13±0.78	26.65±1.35	14.88±0.41***	30.69±1.40	24.95±1.23

All values are expressed as mean ± S.E.M (n = 6 mice per group) and statistical analysis was carried out using One-way ANOVA followed by Dunnett's test of significance.
***P*< 0.01, when treatment group is compared with control
** *P*< 0.001, when treatment group is compared with control
****P*< 0.0001, when treatment group is compared with control

DISCUSSION

RT4
SPECIES: Human (Homo sapiens),

CELL TYPE: Epithelial,

TISSUE OF ORIGIN: Bladder, derived from explants of a recurring papillary tumour of the bladder Bladder cancer is the sixth most common cancer in the United States after lung cancer, prostate cancer, breast cancer, colon cancer, and melanoma. It is the fourth most common cancer in men and the eleventh most common cancer in women. Of the roughly 82,000 new cases reported annually, which about 62,000 are in men and about 20,000 are in women. Of the roughly 17,000 annual deaths, more than 12,000 are in men and fewer than 5,000 are in women.[14,15] Bladder cancer is one of the most fatal urinary malignant tumors in humans, with high incidence, high recurrence rate, high morbidity & mortality and recurrence metastasis rate. The current treatment options like hormone therapy, radiotherapy, chemotherapy are into practice, but severe side effects limit its clinical use.[16,17]

L929
BIOLOGICAL SOURCE: Adipose from mouse,

MORPHOLOGY: Fibroblast

TISSUE OF ORIGIN: Subcutaneous connective tissue; areolar and adipose
Fibroblasts represent a heterogeneous cell subset differentiated from embryonic mesenchymal cells, which are spindle or polygonal in shape with round or oval nucleus, and lack epithelial and leukocyte lineage markers.[18] Fibroblasts are widely distributed in various tissues and actively involved in tissue damage repair, remodeling and fibrosis. Fibroblasts stimulated by degeneration, necrosis or tissue injury are demonstrated to participate in inflammatory response and immune regulation by secreting factors such as nitric oxide (NO), interleukin-1b (IL-1b) and IL-6, and facilitate wound healing by infiltrating to the damaged site, gradually replacing white blood cells.[19] Fibroblasts are also involved in tissue remodeling in the late stage of inflammation through cell proliferation, adhesion, migration and collagen synthesis, while abnormal activation of fibroblasts may result in scar formation and tissue fibrosis.[20] Therefore, the regulation of fibroblast activities is crucial to the inflammatory process and outcome. Fibroblasts are traditionally defined as the cells that produce collagens and are considered to be the primary source of most extracellular matrix components. They play a critical role in regulating the turnover of extracellular matrix and play an important part in wound healing. Fibroblasts are able to differentiate to myofibroblasts, specialized cells that possess a contractile phenotype with α-smooth muscle actin expression.Myofibroblasts are responsible for the generation of the contraction forces that allow wound contraction during wound healing process.
EAC cells are originally derived from murine mammary tissue, and they are undifferentiated, and hyper-diploid in nature. EAC cells are aggressive with 100% tumorigenic ability.[21]
EAC form free neoplastic cells in peritoneal ascites fluid of mice and can be passaged and maintained in peritoneal cavity of the mice and rats.[22]

LEMON OIL

Citrus (such as lemon) is one of the necessary fruits of high medicinal value and has long been the basis of commonly used traditional medicines in many Asian countries. The anticancer activity of lemon oil is mainly due to major flavonoid Hesperidin and considerable amount of research has been carried out

on the anticancer activities of hesperidin and its aglyconehesperetin. On EAC model lemon oil showed decrease in tumor volume and weight. However the hemoglobin level and serum protein level were slightly increased i.e., statistically not significant compared to control. The above results were compared with 5-FU as a standard and these results are supportive of the fact that lemon oil has potential tumor suppressor activity. The cytotoxic and antitumor activity of lemon oil on various other cancers with significant results were reported.

Naz H *et al*. reported that apoptosis induction and cell cycle arrest are among the most important mechanism of hesperidin against cancer cells. Hesperidin has multifaceted roles such as inhibition of the invasion, proliferation, metastasis, migration, and angiogenesis of cancer cells, along with apoptotic and autophagic induction.[23]Afshari K *et al.* reported that hesperidin showed significant anticancerous potential against numerous cancer types *in vivo*, including prostate, breast, colon, and lymphoma. Swiss albino mice, Balb/c nude mice, and Wistar rat models are commonly used to investigate the chemotherapeutic potential of hesperidin.[24]Suzui M *et al*. reported chemopreventive potential of hesperidin against an inducer of colon carcinogenesis (azoxymethane - AOM) in mice model. The mechanism of hesperidin at molecular level induces apoptosis and cell cycle arrest in AOM that produce mouse colon carcinogenesis via inhibiting Phosphoinositide 3-kinase/Protein kinase B (P13K/ Akt) pathway.[25] Zhang J *et al.* reported that hesperetin inhibits the proliferation and induces the apoptosis of hepatocellular carcinoma through triggering the activation of the mitochondrial pathway by rising levels of intracellular reactive oxygen species (ROS), Ca2+ and ATP on male Balb/c-nu/nu nude mice. Another study showed that hesperetin suppressed efficiently the growth of xenografttumors in mice model of gastric cancer.[26]

JASMINE OIL

Literature survey reported that anticancer activity of jasmine oil is mainly due to major alkaloid "Jasmine" that showed stronger cytotoxic activities towards PC-3 (Human prostate cancer) cell line and A549 (human lung adenocarcinoma) cell line.jasmonates can suppress the proliferation of various cancer cells and induce their death. On EAC model jasmine oil didn't show significant reduction in tumor volume and tumor weight but showed significant increase in hemoglobin levels with significant reduction in eosinophils count were reported. The serum protein levels didn't show significant resultscompared to control. These above facts indicate that the anticancer effect of jasmine oil were not that significant on EAC cell lines compared to lemon oil. All these above results were compared with 5-FU as a standard. The researchers have proved the cytotoxic and antitumor activity of jasmine oil on various other cancer cell lines with significant results.

Fingrut O &Flescher E reported that Jasmonates and some of their synthetic derivatives were shown to inhibit the proliferation and to induce cell death in various human and murine cancer cell lines, including breast, prostate, melanoma, lymphoblastic leukemia and lymphoma cells.[27]Recently, Yeruva*et al*. have demonstrated similar anti-cancer effects of jasmonates on both prostate and breast cancers. Study on breast cancer cells MJ inhibited the growth of MDA-MB-435 and MCF-7 cells, induced by cell-cycle arrest and apoptosis.[28] Tong QS *et.al.* reported that MJ was shown to suppress the growth of cultured neuroblastoma cells in association with down regulation of proliferating cell nuclear antigen (PCNA), and to induce apoptosis via modulation of expression of two anti-apoptotic proteins, XIAP (X-linked inhibitor of apoptosis protein) and surviving.[29]

CONCLUSION

Lemon oil has showed significant cytotoxic activity towards mouse fibroblast cell line (L929) and urinary bladder cell line (RT4) compared to jasmine oil. Lemon oil at a concentration of 1000 µg/ml, 500 µg/ml, 250 µg/ml & 125µg/ml lemon oil has showed statistically significant (***P< 0.0001) anticancer activity towards L929 cell line and towards RT4 cell line, lemon oil showed statistically significant (***P < 0.0001) anticancer activity only at an concentration of 1000 µg/ml. On other hand, jasmine oil at concentration of500 µg/ml, 250 µg/ml and 125µg/ml showed statistically significant (** P < 0.001) anticancer activity towards RT4 cell line but insignificant towards L929 cell line.(Lemon oil > Jasmine oil).On EAC inoculated mice,lemon oil at a dose of 400mg/kg showed significant reduction in the tumor volume as well as tumor weight compared to jasmine oil. Both the essential oils showed significant increase in MST and % ILS in all treated groups compared to standard. The hemoglobin level was increased by jasmine oil compared to lemon oil. (Jasmine oil >Lemon oil).

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