ISSN 2063-5346 *Section A-Research paper*

ANTITUMOR AND ANTIOXIDANT ACTIVITY OF VOLATILE OILS OF LEAVES OF *CITRUS AURANTIUM* AND *CITRUS SINENSIS* AGAINST DALTON'S ASCITES LYMPHOMA MEDIATED TUMOUR IN MICE

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Article History: **Received:** 01.02.2023 **Revised:** 07.03.2023 **Accepted:** 10.04.2023

Abstract

Introduction: Cancer prevention and treatment are critical today. The best efforts are being made to combat this disease through multidisciplinary scientific investigations.

Background and purpose: The current study sought to assess the antitumor effects of volatile oils extracted from the leaves of *Citrus aurantium* and *Citrus sinensis*, as well as the antioxidant enzyme levels.

Materials and methods: The volatile oils isolated from the leaves of *Citrus aurantium* and *Citrus sinensis* at 25mg/kg and 50mg/kg in an emusion form using acacia gum were tested for antitumor activity in DAL (Dalton's Ascites Lymphoma) bearing swiss albino mice. The tumor cell count, body weight, percentage of increased life span, liver antioxidant enzymes, and histopathological changes in the liver and kidney were studied to interpret the results. As a positive control, 5 Flurouracil at 20mg/kg was used as standard.

Results: The current study demonstrates the potency of volatile oil from *Citrus aurantium* (VOCA) and *Citrus sinensis* (VOCS) in vivo antitumor activity without significant changes in the toxicity parameters evaluated. It was discovered that VOCA and VOCS exerted a dose-dependent decrease in cell viability and increased protection against experimental animals from the deleterious effect of DAL-induced tumor in mice.

Conclusion: It has been demonstrated that VOCA and VOCS have potent antitumor activity.

Key words:*Citrus aurantium, Citrus sinensis,* Volatile oil, Antitumour, DAL cell lines.

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INTRODUCTION

Combating cancer is of paramount importance today. Multidisciplinary scientific investigations are making the best efforts to combat this disease, but the sure-shot, perfect cure is yet to be brought into world medicine. An alternative solution to western medicine embodied with severe side effects is the use of medicinal plant preparations to arrest the insidious nature of the disease. Of the 92 anticancer drugs commercially available prior to 1983 in the United States, approved worldwide between 1983 and 1994, approximately 62% can be related to natural origins[1]. Many herbs have been evaluated in clinical studies and are currently being investigated phytochemicaly to understand their tumoricidal actions against various cancers[2]. The rich and diverse plant sources of India are likely to provide effective anticancer agents. One of the best approaches in the search for anticancer agents from plant resources is the selection of plants based on ethnomedical leads[3]. Flavonoids have important effects on cancer chemoprevention and chemotherapy. Flavonoids are a group of about 4000 naturally polyphenolic compounds, found universally in plant origin. According to the differences in functional groups and their relative positions of the 15-carbon skeleton (aglycons), flavonoids are classified into several subgroups including the following: flavone, flavanone, flavonol, isoflavonoid, anthocyanidin and chalcones[4]. Flavonoids are widely present in the genus Citrus (family Rutaceae)[5]. Many mechanisms of action of flavonoids have been identified, including carcinogen inactivation, antiproliferation, cell cycle arrest, induction of apoptosis and differentiation, inhibition of angiogenesis, antioxidation and reversal of multidrug resistance or a combination of these mechanisms[6]. Fruits by-products such as seeds, peels, stems, barks and leaves usually been thrown into an environment which causes serious disposal problem in food and agriculture industries.

Therefore, extensive researches have been carried out worldwide in order to minimize the above stated problem. *Citrus sinensis* (Orange, Rutaceae) fruit peels are beneficial to human health. Orange peels have been used in food, drug and cosmetic products. However, the overall demand of orange peels is of insignificant as applications have not been widely explored and recognized. The major constituents of orange peels include flavonoids such as polymethoxy flavonoids; terpenoids, such as limenene and linalool. These flavonoids are found to have antioxidant[7], antimutagenic[8], antiallergic, anti-inflammatory and antimicrobial[9] activities. The objective of the present study was to evaluate in vivo antitumor effects of hydroalcoholic extract of *Citrus aurantium*and *Citrus sinensis* including the estimation of antioxidant enzyme levels.

MATERIALS AND METHODS

Experimental animals

Swiss albino mice of both sex and weighing 20–25 g were used in the current study. Animals at the animal house facility were transferred into separate polyethylene cages. One week before experiments, the animals were acclimatized to the laboratory environment.The animals were housed in a sterile setting (temperature: 24°C ± 2°C, relative humidity: 55%–65%, and 12 h dark/light rhythm) in polypropylene cages containing sterile paddy husk as bedding material with a maximum of six animals in a cage and fed standard pellets and *ad libitum* water. Protocols used in the animal model study were carried out with the prior approval of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India with approval number: CBLRC/IAEC/09/01/2021.

Chemicals

The chemicals and solvents used in the study were of the highest purity and analytical reagents grade. The chemicals were purchased from SD Fine Chem., Himedia and Sigma, India.

Induction of lymphoma

Dalton's Ascites Lymphoma (DAL) cells were obtained from Amala Cancer Research Institute, Kerala, India. The cells were maintained in vivo in Swiss albino mice by intraperitoneal transplantation of 1×10^6 cells/mouse. The DAL cells aspirated from the peritoneal cavity of the mice were washed with saline and given intraperitoneally to the experimental animals to develop ascitictumors.

Experimental protocol

Group I containing 6 animals, served as the normal control, for which inoculation of tumor cells was not done. The remaining animals were inoculated with DAL (1×10^6 cells/mouse) and divided into 6 groups containing 6 mice in each group. Group II, served as the tumor control. Groups I and II received normal saline 5ml/kg through orally. Group III, which served as a positive control, was treated with 5-Fluro uracil (5-FU) at the dose of 20 mg/kg body weight intraperitoneally.Groups IV which served as a

treatment group, was treated with volatile oil emulsion of *Citrus aurantium* (VOCA) at the dose of 25 mg/kg body weight orally. Groups V served as a treatment group, was treated with volatile oil emulsionof *Citrus aurantium* (VOCA) using acacia gumat the dose of 50 mg/kg body weight orally.Groups VI served as a treatment group treated with volatile oil emusionof *Citrus sinensis* (VOCS) at the dose of 25 mg/kg body weight orally.Groups VII served as a treatment group treated with volatile oil emulsion of *Citrus sinensis* (VOCS) at the dose of 50 mg/kg body weight orally.

All the drugs and test samples were given orally at 24 h after tumor inoculation and continued once daily for 14 days. On the 15th day, six animals from each group were anaesthetized slightly with anesthetic ether and blood was collected from retro-orbital puncture. The hematological parameters like WBC, RBC, hemoglobin (Hgb), and platelets (PLT) were estimated by a cell analyzer (Medonic CA 530; Boule Medical, AB). The remaining blood was centrifuged and serum was used for the estimation of hepatoprotective parameters like aspartate amino transferase (ASAT), alanine amino transferase (ALAT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), triglycerides (TGL), creatinine (CR), albumin and total protein (TP) by using standard Kits.

Cancer cell count

The fluid (0.1 ml) from the peritoneal cavity of each mouse was withdrawn by sterile syringe and diluted with 0.8 ml of sterile Phosphate Buffer Solution and 0.1 ml of tryphan blue (0.1 mg/ml) and the total number of the living cells was counted using a hemocytometer. The cell count was calculated by the formula: Cell Count = (No. of cells in dilution)/(Area x thickness of liquid film).

Body weight

All the mice were weighed from the beginning to the fifteenth day of the study. An average increase in body weight on the fifteenth day was determined.

Percentage increase in lifespan

Percentage increase in lifespan (ILS) was calculated by the formula: % ILS = (Lifespan of treated group)/(Lifespan of control group) -1 ×100.

Estimation of Lipid peroxidation, Superoxide dismutase, Catalase

The thiobarbituric acid reactive substances (TBARS) in the cell lysate tissues were measured as per the method reported earlier[10]. The TBARS content was expressed in micromole/milligram (mmoles/mg) of protein. The SOD activity in cell lysate was determined as per the method followed earlier [11]. Enzyme activity was expressed as 1 Unit = 50% inhibition/minute/milligram of protein. The CAT activity in cell lysate was assayed as per the method reported earlier [12]. CAT was expressed in terms of micromoles of hydrogen peroxide decomposed/minute/ milligram of protein.

Histopathological study

The liver and kidney tissue samples collected were fixed in 10% formalin solution. After fixation, the tissues were embedded in paraffin and sections cut at 5 µm to later be stained with hematoxylin and eosin. The sections were then examined under light microscope and photographed.

Statistical analysis

The values are represented as mean ±SD. The experimental data were assessed by the One-way Analysis of Variance (ANOVA) method followed by Newmann keuls multiple comparison. The results were considered to be statistically significant when the $P < 0.01$.

RESULTS

Table 1. Effect of volatile oils of *Citrus aurantium* and *Citrus sinensis* on hematological parameters of DAL bearing mice

Groups	Total WBC (1×10^3 /mm^3)	RBC (1×10^6 /mm^3)	Hgb(g/dl)	PLT (10^3 /mm^3
Normal control	10.85±0.84	4.42±0.48	12.85±0.72	3.55±0.48
Disease control	14.38±1.23*a	2.40±0.25*a	7.24±0.38*a	1.84±0.22*a
Standard group (5FU 20 mg/kg)	11.34±0.95*b	4.15±0.38*b	11.55±0.48*b	2.95±0.34*b
Treatment group-1 (VOCA 25mg/kg)	12.55±1.05*b	3.44±0.30*b	10.35±0.45*b	2.42±0.40*b
Treatment group-2 (VOCA 50mg/kg)	12.10±0.96*b	3.78±0.37*b	10.78±0.64*b	2.67±0.47*b
Treatment group-3 (VOCS 25mg/kg)	12.30±1.00*b	3.56±0.34*b	10.58±0.54*b	2.51±0.44*b
Treatment group-4 (VOCS 50mg/kg)	11.88±0.92*b	3.85±0.40*b	11.02±0.69*b	2.76±0.50*b

All values are expressed as mean ± SEM for 6 animals in each group.*a – Values are significantly different from control (G_1) at $P < 0.01$. *b – Values are significantly different from cancer control (G_2) at $P < 0.01$.

Table 2: Effect of volatile oils of *Citrus aurantium* and *Citrus sinensis* on body weight, packed cell volume, viable cell count, and percent increased lifespan of tumor-induced mice

Groups	Increase in body weight (g)	Packed cell volume (ml)	Viable cell count (cells ×106 /mL)	Percent ILS Lifespan
Normal control	2.47±0.55	21.20±1.74	1.84±0.26	100%
Diseased control	7.77±1.15*a	33.35±2.95*a	2.78±0.34*a	46%
Standard group (5FU 20 mg/kg)	3.24±0.38*b	18.48±1.65*b	1.42±0.23*b	93%
Treatment group-1 (VOCA 25mg/kg)	4.17±0.47*b	23.75±1.90*b	2.05±0.30*b	68%
Treatment group-2 (VOCA 50mg/kg)	3.95±0.42*b	21.20±1.74*b	1.84±0.26*b	74%
Treatment group-3 (VOCS 25mg/kg)	4.24±0.52*b	23.45±1.80*b	2.12±0.34*b	72%
Treatment group-4 (VOCS 50mg/kg)	3.84±0.39*b	20.25±1.65*b	1.73±0.22*b	77%

All values are expressed as mean ± SEM for 6 animals in each group.*a – Values are significantly different from control (G_1) at $P < 0.01$. *b – Values are significantly different from cancer control (G_2) at $P < 0.01$.

Table 3. Effect of volatile oils of *Citrus aurantium* and *Citrus sinensis* on serum biochemical parameters of tumor-induced mice

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	LDH (U/I)	TGL (mg/dl)	CR (mg/dl)	Albumin (mg/dl)	TP (g/dl)
Normal control	92.30 ± 3.85	48.60 ± 2.12	80.40 ± 2.15	87.45 ± 3.05	154.58 ± 5.74	1.03 ± 0.10	3.70 ± 0.65	6.74 ± 0.48
Disease control	155.45 ± 5.76*a	88.85 ± 2.25*a	135.22 ± 3.45*a	119.85 ± 4.55*a	224.68 ± 7.65*a	1.58 ± 0.22*a	2.15 ± 0.14*a	12.10 ± 1.24*a
Standard group (5-FU 20 mg/kg)	106.20 ± 4.10*b	52.20 ± 1.75*b	93.70 ± 2.78*b	93.65 ± 3.38*b	170.10 ± 5.95*b	1.14 ± 0.14*b	3.40 ± 0.42*b	7.65 ± 0.82*b
Treatment group-1 (VOCA 25mg/kg)	123.32 ± 4.35*b	60.25 ± 1.88*b	101.12 ± 3.04*b	105.24 ± 3.90*b	188.15 ± 6.26*b	1.27 ± 0.19*b	2.90 ± 0.28*b	8.45 ± 0.98*b
Treatment group-2 (VOCA 50mg/kg)	114.15 ± 4.15*b	57.38 ± 1.72 *b	97.20 ± 2.96*b	99.37 ± 3.55*b	180.35 ± 5.98*b	1.20 ± 0.16*b	3.17 ± 0.32 *b	8.10 ± 0.88*b
Treatment group-3 (VOCS 25mg/kg)	121.24 ± 4.28*b	62.75 ± 1.96*b	104.40 ± 3.36*b	103.16 ± 3.72*b	182.70 ± 6.12*b	1.30 ± 0.22*b	2.98 ± 0.30*b	8.23 ± 0.90*b
Treatment group-4(VOCS 50mg/kg)	111.30 ± 4.10*b	55.45 ± 1.82 *b	99.70 ± 3.15*b	97.45 ± 3.36*b	177.30 ± 5.84*b	1.24 ± 0.19*b	3.24 ± 0.35 *b	7.90 ± 0.84*b

All values are expressed as mean ± SEM for 6 animals in each group.*a – Values are significantly different from control (G_1) at $P < 0.01$. *b – Values are significantly different from cancer control (G_2) at $P < 0.01$

Table 4: Effect of VOCA and VOCS on lipid peroxidation, SOD, and CAT in DAL bearing mice

Groups	MDA (µM/g of protein)	SOD (IU/mg of protein)	CAT (IU/min/mg of protein
Normal control	163±4.65	7.8±0.45	35.6±2.15
Disease control	488±10.95 *a	3.7±0.24 *a	9.8±0.85 *a
Standard group (5-FU 20 mg/kg)	250±6.70*b	6.9±0.38*b	27.4±1.85*b
Treatment group-1 (VOCA 25mg/kg)	315±7.30*b	6.1±0.27*b	20.5±1.33*b
Treatment group-2 (VOCA 50mg/kg)	295±6.90*b	5.3±0.20*b	23.7±1.47*b
Treatment group-3 (VOCS 25mg/kg)	326±7.42*b	6.4±0.32*b	21.8±1.40*b
Treatment group-4 (VOCS 50mg/kg)	287±6.45*b	5.8±0.25*b	24.1±1.54*b

All values are expressed as mean ± SEM for 6 animals in each group.*a – Values are significantly different from control (G_1) at $P < 0.01$. *b – Values are significantly different from cancer control (G_2) at $P < 0.01$.

RESULTS

Effect on tumor growth

The average lifespan of animals in DALtumor control group was found to be 46% (Table 2). The average lifespan of animals treated with VOCA and VOCS at the doses of 25 and 50 mg/kg body weight was found to be 68,74,72 and 77%, respectively, whereas the average lifespan of 5-FU-treated group was found to be 93%. An increase in packed cell volume is observed inDAL control mice over the extract treated group. VOCA and VOCS treated groups showed a significant ($p<0.01$) reduction in the packed cell volume.

Similarly, VOCA and VOCS exhibited significant($p<0.01$) decrease in the viable cell count at the dose of 50 mg/kg than ($p<0.01$) VOCA and VOCS-treated group at the dose of 25 mg/kg. Moreover the antitumor nature of VOCA and VOCS at a doseof 25 and 50 mg/kg was also evident by the significant ($p<0.01$) reduction in percent increase in body weight of animal treated with VOCA and VOCS at a dose of 25 and 50 mg/kg body weight when compared to DALtumor bearing mice. All these results indicate that VOCA and VOCS hassignificant ($p<0.01$) activity to inhibit tumor growth induced by DAL cell line.

Effect on hematological parameters

As shown in Table 2, RBC, Hgb and PLTwere decreased and WBC count was significantly increased in the DAL control group as compared to the normal group. Treatment with VOCA and VOCS at both the doses significantly increased the Hgb content, RBC, PLT, and significantly decreased the WBC count to about normal levels. All these results suggest the anticancer nature of the extract. However, the standard 5-FU at the dose of 20 mg/kg body weight produced better results in all these parameters.

Effect on biochemical parameters:

The inoculation of DAL cells caused significant decreases in the levels of albumin ,whereassignificant increases in the levels of AST,ALT,ALP, LDH, CR and TGL in the serum of tumor control animals, when compared to the normal group. The treatment with VOCA and VOCSat 25 and 50 mg/kg body weight reversed these changes towards the normal levels (Table 3). Most of the values were found to be significant. The treatment with standard 5-FU also gave similar results.

Effect on superoxide dismutase, catalase, and lipid peroxidation

Superoxide dismutase and catalase activities were reduced significantly ($P < 0.01$) in the DAL control groups compared to that of the normal group. Treatment of both the VOCA and VOCS group significantly ($P < 0.01$) restored the SOD level to the normal value, when compared with the DAL control group. Similarly, administration of VOCA and VOCS at 25 and 50 mg/kg significantly ($P < 0.01$) recovered the CAT level to the normal value when compared with the DAL control group [Table 4].

The TBARS levels expressed as malondialdehyde (MDA) were significantly ($P < 0.01$) increased in the DAL control animals when compared to that of the normal control group. Treatment with VOCA and VOCS at 25 and 50 mg/kg body weight significantly ($P < 0.01$) reduced the MDA levels when compared with the DAL control group.

Histopathological observations

Histological examination of the liver and kidney tissues under a light microscope was done to observe the effects of VOCA and VOCS on the structural integrity of the cells. The liver of normal animals showed normal histological appearance (Fig. 1). The tumor control animal liver showed slight enlargement of hepatocytes, dilated sinusoidal spaces containing lymphocytes, and portal triads showing collections of lymphocytes (Fig. 2). The animals treated with standard 5-FU at 20 mg/kg body weight and VOCAat 25 and 50 mg/kg body weight exhibited almost normal histological appearance of liver cells, except for a few lymphocytic collections in the portal area (Figs. 3). The animals treated with VOCS at 25 and 50 mg/kg body weight also showed normal histology with no lymphocytes in the portal area, indicating its potent hepatoprotective action when compared to standard 5-FU treatment (Fig. 4-7). The kidney of normal animals showed normal histological appearance (Fig. 8). The tumor control animals exhibited atrophied glomeruli and dilated renal tubules (Fig. 9). However, the kidney of VOCA and VOCS treatment at both the doses and standard 5-FU treatment showed normal histological appearance. These findings clearly

indicate that the liver and kidney tissues that were damaged by DAL inoculation showed recovery with VOCA and VOCS and 5-FU treatments (Fig. 10-14).

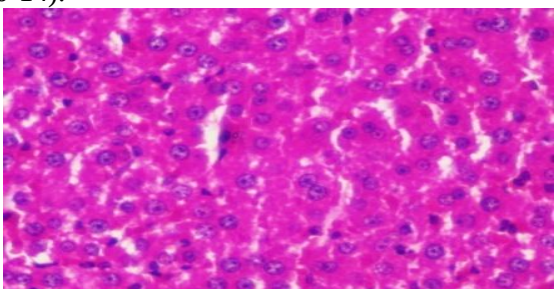

Fig.1 Normal Control Liver

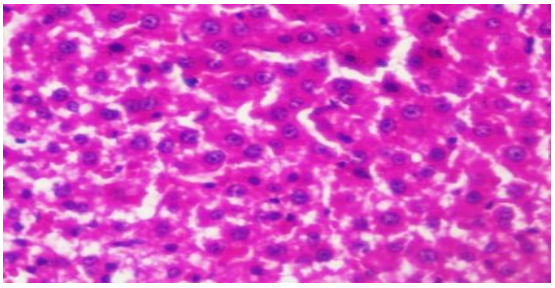

Fig.2 DALInduced Toxic Control

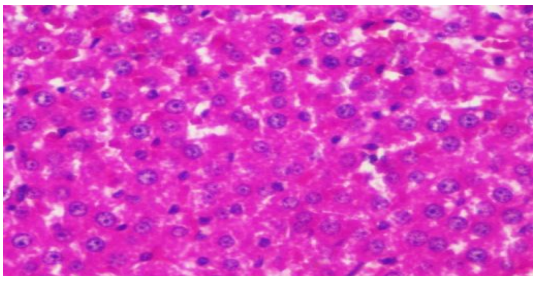

Fig.3 Standard Control 5FU 20mg/Kg

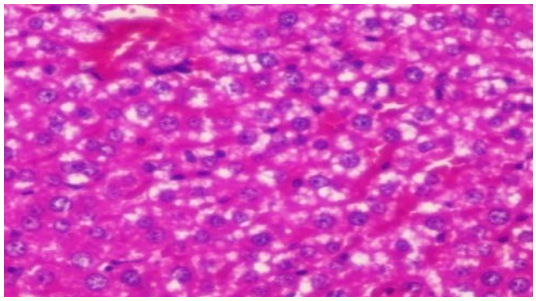

Fig.4 Treatment Control VOCA 25mg/Kg

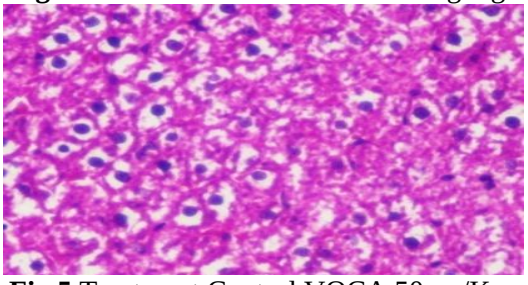

Fig.5 Treatment Control VOCA 50mg/Kg

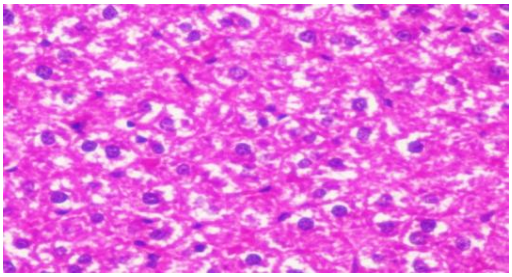

Fig.6 Treatment Control VOCS 25mg/Kg

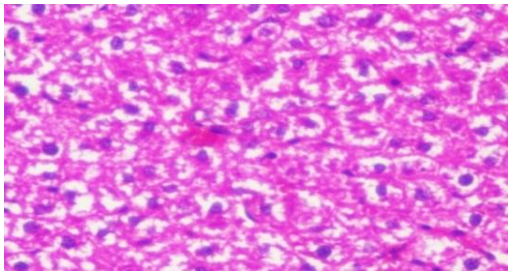

Fig.7 Treatment Control VOCS 50mg/Kg

The liver of normal animals showed normal histological appearance (Fig. 1). The tumor control animal liver showed slight enlargement of hepatocytes, dilated sinusoidal spaces containing lymphocytes, and portal triads showing collections of lymphocytes (Fig. 2). The animals treated with standard 5-FU at 20 mg/kg body weight and VOCA at 25 and 50 mg/kg body weight exhibited almost normal histological appearance of liver cells, except for a few lymphocytic collections in the portal area (Figs. 3 to 5). The animals treated with VOCS at 25 and 50 mg/kg body weight also showed normal histology with no lymphocytes in the portal area, indicating its potent hepatoprotective action when compared to standard 5-FU treatment (Fig. 6 and 7).

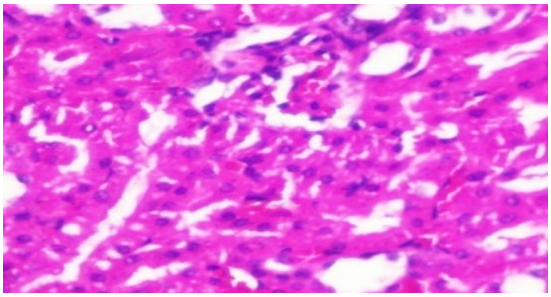

Fig.1 Normal Control Kidney

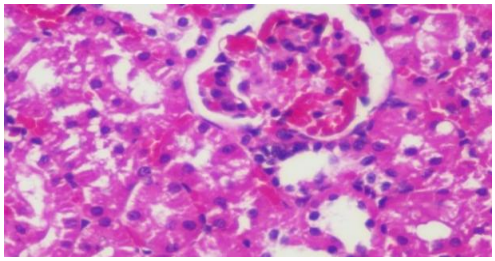

Fig.2 DAL Induced Toxic Control

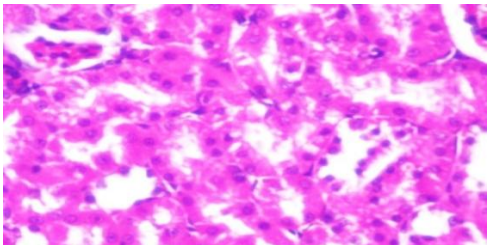

Fig.3 Standard Control 5-FU 20mg/Kg

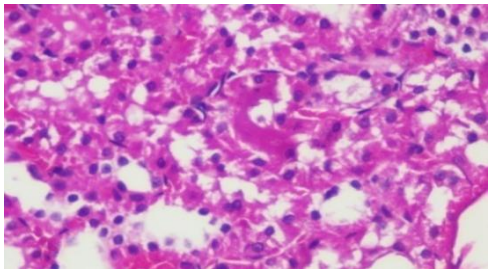

Fig.4 Treatment Control VOCA 25mg/Kg

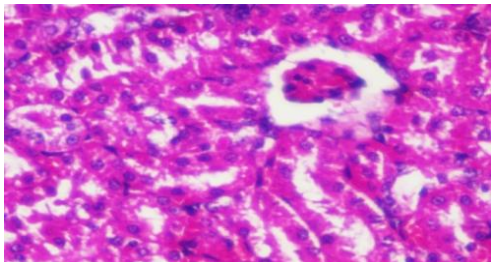

Fig.5 Treatment Control VOCA 50mg/Kg

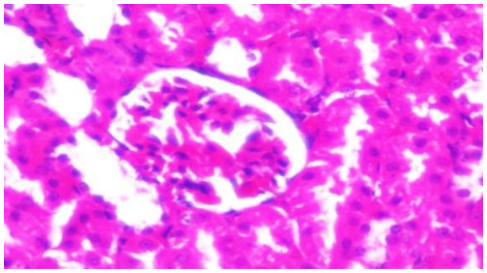

Fig.6 Treatment Control VOCS 25mg/Kg

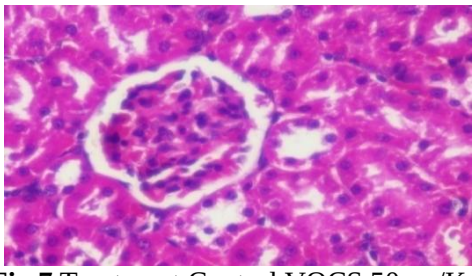

Fig.7 Treatment Control VOCS 50mg/Kg

The kidney of normal animals showed normal histological appearance (Fig. 1).The tumor control animals exhibited atrophied glomeruli and dilated renal tubules (Fig. 2). However, the kidney of VOCA and VOCS treatment at both the doses and standard 5-FU treatment showed normal histological appearance. These findings clearly indicate that the kidneytissues that were damaged by DAL inoculation showed recovery with VOCA and VOCS and 5-FU treatments (Fig. 3 to 7).

DISCUSSION

In the present study, intraperitoneal inoculation of DAL cells in the mice produced an enormous increase in the cancer cell count, which indicated that there is progression of cancer in the animals. Ascetic fluid is the direct nutritional source for tumor cells and, therefore, a rapid increase in ascetic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells[13,14]. The reliable criterion for judging the anticancer effect of volatile oil from VOCA and VOCS is reduction in viable cell count towards normal. It may be due to the VOCA and VOCS stimulate the growth and activity of immune cells by the production of Interleukins, which target tumor cells and cause lysis of the tumor cells by indirect cytotoxic mechanism. Furthermore, the reduced PCV and increased survival time of the mice suggest that the VOCA and VOCS might have exerted a delay in vascular permeability to the cells[15].

The reliable measure for assessing the value of anticancer drug is increasing the lifespan of the tumor bearing animal[16]. It can, therefore, be understood that VOCA and VOCS increased the lifespan of DAL bearing mice which was due to the prevention of tumor development. Thus VOCA and VOCS has antitumor activity against DAL-bearing mice at a dose of 25 and 50 mg/kgbody weight.Myelosupression and anemia are major problems that are encountered in the treatment of cancer chemotherapy[17,18]. Anemia occurring in tumor bearing mice is mainly due to reduction in erythrocytes or hemoglobin and this may happen either due to iron deficiency or due to hemolytic or myelopathic conditions[19,20]. In the present study, the results indicate that VOCA and VOCS significantly increased the erythrocyte count and hemoglobin level when compared to those of DAL controlled mice. Moreover, the WBC count had decreased when compared to that of DAL-controlled mice.These parameters show that VOCA and VOCS shows less toxic effect tothe hemopoietic system and reasonably had selective affinity to the tumor cell and hence it could maintain the normal hematological profile.

It was reported that the presence of tumor in the human body or in the experimental animals is known to affect may function of the liver. The significantly elevated level of AST, ALT, ALP, LDH, CR and TGL in serum of tumor inoculated animal indicated liver damage and loss of functional integrity of cell membrane. The significant reversal of these changes towards the

normal by VOCA and VOCSat a dose of 25 mg/kg and 50mg/g body weight treatments.

In the present study, the biochemical examination of DAL inoculated animals showed marked changes indicating the toxic effect of the tumor. VOCA and VOCS significantly reducedviability of tumor cells and packed cell volume, and normalized the hematological profile and serum biochemical parameters, raising the lifespan of the treated group as compared with those of DAL control mice. Also, the group treated with VOCA and VOCS improved the enzymatic and nonenzymatic antioxidant systems. Decrease of lipid peroxidation and augmentation of SOD and CAT in VOCA and VOCS treated mice showed its potential as an inhibitor of DAL-induced intracellular oxidative stress. Thus, VOCA and VOCS demonstrated remarkable in vivo antitumor activity against DAL in mice plausibly by attributing lipid peroxidation and increasing endogenous antioxidant systems.

CONCLUSION

The present study demonstrates the potency of volatile oil from VOCA and VOCS showing *in-vivo* antitumour activity without major changes in toxicity parameters evaluated. It was found a dramatic decrease in cell viability and increased protection against experimental animals from the deleterious effect of DAL induced tumour in mice exerted by VOCA and VOCS in a dose-dependent manner. Hence, it is proved that VOCA and VOCS possessed potent antitumour activity. This indicates that the volatile from VOCA and VOCS may potentially provide better bioactive compounds with substantial anti-proliferative characteristics that could be useful in primary healthcare. However, to support the above, further investigations are being carried out to elucidate the exact mechanism of action and its metabolism studies.

Authors Contribution

All the authors has equally contributed to make the research and its findings.

Conflict of Interest: None declared

ACKNOWLEDGEMENT

The authors are very much greatful to the Cap bio lab for ethical clearance and conduct of invivo antitumour studies. The authors also want to thank the research committee, College of Pharmacy, Vinayaka Mission Research Foundation (VMRF) (DU), Salem.

REFERENCES

1. Gordon MC, Newman DJ. Natural product drug discovery in the next millennium. Pharm Biol. 2001; 39: 8–17.
2. Premalatha B, Rajgopal G. Cancer – an ayurvedicperspective.Pharmacol Res. 2005; 51: 19-30.
3. Spiridon KE. Terrestrial plant-derived anticancer agents and plants used in anticancer research. Crit Rev Plant Sci. 2006; 25: 79–113.
4. Harborne JB. Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis. 3rd ed. India, New Delhi: Springer Pvt. Ltd. 1998.
5. Obdulio BG, Julian C, Francisco RM, Ana O, Jose AD. Uses and properties of Citrus flavonoids. J Agric Food Chem. 1997; 45(12): 4505-15.
6. Manthey JA, Guthrie N, Grohmann K. Biological properties of Citrus flavonoids pertaining to cancer and inflammation. Curr Med Chem. 2001; 8(2): 135-53.
7. Lai CS, Li S, Miyauchi Y, et al. Potent anti-cancer effects of citrus peel flavonoids in human prostate xenograft tumors. Food Funct. 2013; 4(6): 944-9.
8. Hosseinimehr SJ, Karami M. Citrus extract modulates genotoxicity induced by cyclophosphamide in mice bone marrow cells. J Pharm Pharmacol. 2005; 5(4): 505-9.
9. Hamdan D, Ashour ML, Mulyaningsih S, et al. Chemical composition of the essential oils of variegated pink-fleshed lemon (Citrus x limon L. Burm. f.) and their anti-inflammatory and antimicrobial activities. Z Naturforsch. 2013; 68(7- 8): 275-84.
10. Ohkawa H, Onishi N, Yagi K. Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351‑ 8.
11. Kakkar P, Das B, Vishwanath PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys. 1984; 21: 130‑ 2.
12. Aebi H. Catalase. In: Packer L, editor. Methods in Enzymatic Analysis. York: Academic Press; 1974; 2: 673‑ 84.
13. Pallab KH, Biswakanth K, Asis B et al., Antitumor activity of Sansevieriarox burghiana rhizome against Ehrlich ascites carcinomain mice. Pharm Biol. 2010; 48:1337–43.
14. Prasad SB, Giri A. Antitumor effect of cisplatin against murineascites Dalton's lymphoma. Indian J ExpBiol. 1994; 32: 155–62.
15. Bhist M, Bist SS, Dhasmana DC. Biological response modifiers: Current use and future prospects in cancer therapy. Indian Journal of Cancer. 2010; 47(4): 443-451.

16. Clarkson D, Burchneal JH. Preliminary screening of antineoplastic drugs. Prog Clin Cancer. 1965; 1: 625–9.
17. Hogland HC. Hematological complication of cancer chemotherapy.Semin Oncol. 1982; 9: 95–102.
18. Prince VE, Greenfield RE. Anemia in cancer. In: Grensstein JP, Haddaw A, eds. Advances in Cancer Research, vol. V. New York:Academic Press, 1958; 199–200.
19. Fenninger LD, Mider GB. Energy and nitrogen metabolismin cancer. Adv Cancer Res. 1954; 2: 229–53.
20. Sarita K, Kiranmai M, and Prakash Yoganandam G, Pharmacognostical Phytochemical and anticancer studies of *Dendrophthoe falcata* (L.F) Ettingish.(Loranthaceae) growing on the host plant *Azadirachta indica* (Meliaceae), International Journal of Pharma and Bio Sciences, 2013, Vol 4(2): 1010-1018.