RESEARCH ARTICLE

Antioxidant Activity of Essential Oils from *Wedelia chinensis (Osbeck) in vitro* and *in vivo* Lung Cancer Bearing C57BL/6 Mice

A Manjamalai, VM Berlin Grace*

Abstract

Aim: The present investigation was to evaluate the effects of essential oils of *Wedelia chinensis (Osbeck)* on free radicals and *in vivo* antioxidant properties. Methods: Essential oils were extracted using hydro-distillation and compound analysis was performed by GC-MS analysis. Screening for inhibitory activity was conducted by DPPH and OH-scavenging assays. In addition an *in vivo* study was carried out in cell line implanted cancer bearing mice with assessment of levels of catalase, superoxide dismutase, glutathione peroxidase, lipid peroxidation, nitric oxide and reduced glutathione. Finally, lungs were dissected out for histopathology study of metastasis. Results: GC-MS analysis revealed the presence of carvocrol and trans-caryophyllene as the major compounds with 96% comparison with the Wilily and NBS libraries. The essential oil exhibited significant inhibition in DPPH free radical formation. Whereas reducing power and hydroxyl radical scavenging activity are dose dependent. When compared with the standard, it was found that the essential oil has more or less equal activity in scavenging free radicals produced. In the animal studies, the level of antioxidant enzymes catalase, superoxide dismutase and glutathione peroxidase, as well as glutathione, were found to be increased in treated groups whereas lipid peroxidation and nitric oxide were reduced. Histopathology report also shows that the essential oil has a significant combating effect against cancer development. Conclusion: In all the *in vitro* assays, a significant correlation existed between the concentrations of the essential oil and percentage inhibition of free radicals. The *in vivo* studies also has shown a very good antioxidant property for the essential oil during cancer development. From, these results the essential oil can be recommended for treating disease related to free radicals and to prevent cancer development.

Keywords: *Wedelia chinensis (Osbeck)*-free radicals-antioxidant-reducing power-Lung cancer-catalase

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Introduction

Medicinal plants are used by tribal people as folk medicine to treat diabetes mellitus, rheumatism, abdominal disorders and inflammation. Some are even used for treating insect poisoning (Jaishree et al., 2008). Many compounds have been isolated so far from the medicinal plants includes Saponins, Flavonoids, Terpenoids, Triterpenoids and Phenolic compounds etc., and these compounds are reported to be having protective effects due to their chemical properties present in it. In general, free radicals are said to be an atom or molecule that pocesses an unpaired electron and is extremely reactive and it has a capability to engage in a rapid chain reaction that unstabilizes other molecules and generate more free radicals. These free radicals are highly toxic and generate oxidative stress in plants and animals even though; they have an in built wide range of combating mechanism for these free radicals. But, these free radicals mediated toxicity can be effectively eliminated by the compounds from plant origin and many of these activities have been reported already for a wide range of plants with different compounds (Sulekha et al., 2009). However, these free radicals also play a beneficial role *in vivo* for instance production of ATP molecules, Phagocytosis, regulation of cell growth, intercellular signaling and in the synthesis of biological compounds (Alluri et al., 2009). A decade back literature states that the essential oil and various plant extracts have become a great interest, because of their tremendous source of natural products and their efficiency in treating many infectious diseases and also used as an alternative remedies for treating dreadful diseases (Maria et al., 2010). Apart, from the plant extracts that are commonly known, the essential oils are now implicated for various medicinal purposes. Essential oils commonly represent a fraction of plant's composition by which they are used in the food, cosmetic and pharmaceutical industries. The compounds isolated from the essential oils

*Department of Biotechnology, Karunya University, India *For correspondence: berlingracehead@gmail.com*

are used in food preservative and additives and as natural remedies for treating some disorders. Some essential oils appear to exhibit particular medicinal properties that have been claimed to cure organ dysfunction or systemic disorder (Huan et al., 2010). Researchers show a great interest in natural antioxidants especially those with plant origin because of their relatively low side effects (Fariba et al., 2011). Our research group has already reported for the Anti-bacterial (Manjamalai et al., 2010). Anti-fungal and Anti-inflammatory activity (Manjamalai et al., 2012) for the methanolic extract as well as for the essential oil of *Wedelia chinensis (Osbeck)* (Manjamalai et al., 2012). In our present investigation, we have analyzed the antioxidant potential of essential oil of *Wedelia chinensis (Osbeck)* both *in vitro* and *in vivo*.

Materials and Methods

Collection and Authentication of Plants

Fresh leaves of the selected plant *Wedelia chinensis (Osbeck)* having medicinal value were collected from Western Ghats of Siruvani hills of Coimbatore, India. The plant materials were taxonomically identified and authenticated by the Botanical Survey of India and the voucher specimen (**No.BSI/SC/5/23/09-10/TECH.1449**) was retained in our laboratory for future reference.

Extraction of essential oil from Wedelia chinensis (Osbeck)

Extraction of essential oil from *Wedelia chinensis (Osbeck)* was done by Hydro distillation method using Clevenger-type apparatus for 3 hours. Plant material (leaves) was immersed directly in a round bottom flask filled with water, then brought to boil. Vapours were condensed on a cold surface using condenser attached to it. Essential oil gets separated based on difference in density and immiscibility, which was then collected and dried over anhydrous sodium sulphate and stored in vial at low temperature until analysis (Extraction Technologies, 2010).

Essential oil analysis for bioactive compounds

GC-MS analysis was performed using SHIMADZU GC-MS QP 2010 using CARBOWAX capillary column and Helium as carrier gas to quantify the major phytochemical. 0.2μl of essential oil was injected in to the column at the flow rate of 1μl/minute. The injector was operated at 250^{o}C and the oven temperature was programmed as follows; 60^{o}C for 15minutes, then gradually increased to 280^{o}C at 3 minutes. The identification of components were based on comparison of their mass spectra with those of Wiley and NBS libraries and those described by Adams as well as comparison of their retention indices (Christina et al., 2005) .

In Vitro Anti-Oxidant Study

DPPH free radical scavenging activity: The free-radical scavenging activity of the essential oil of *Wedelia chinensis (Osbeck)* was measured as a decrease in the absorbance of methanol solution of DPPH. A stock solution of DPPH (33 mg in 1 L) was prepared in methanol, which gave initial absorbance of 0.49 and 5 ml of this stock solution was added to different concentrations of essential oil (5, 10, 25, 50, 100 µg/ml). After 30 min, the pale pink colour developed was measured at 517 nm and compared with standards (5-100 µg/ml ascorbic acid) (Sreejayan et al., 1996). Scavenging activity was expressed as the percentage inhibition calculated using the following formula: %Anti-radical activity=(Control Absorbance-Sample Absorbance)×100/Control Absorbance.

Hydroxyl radical scavenging activity: The essential oil of *Wedelia chinensis (Osbeck)* with different concentrations were placed in test tubes. To which, 1 ml of iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 ml of 0.018% EDTA, 1 ml of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) and 0.5 ml of 0.22% ascorbic acid were added to each tube. The tubes were capped tightly and heated in a water bath at 80-90°C for 15 min. The reaction was terminated by adding 1 ml of ice-cold TCA (17.5% w/v). 3 ml of Nash reagent (75.0 g of ammonium acetate, 3 ml of glacial acetic acid and 2 ml of acetyl acetone were mixed and distilled water was added to make up total volume of 1 L) was added to each tube, which were left at room temperature for 15 min for colour development. The intensity of the yellow colour formed was measured at 412 nm against blank (Klein et al., 1981) Percentage inhibition was determined by comparing the results of the test and standard compound (Ascorbic acid) by using the formula: %inhibition activity=(Control Absorbance-Sample Absorbance)×100/Control Absorbance.

Reducing power assay: The reducing power of essential oil of *Wedelia chinensis (Osbeck)* was determined by adding 1ml of different concentrations of essential oil (5, 10, 25, 50, 100 µg/ml) with 2.5 ml of PBS and 2.5 ml ferrous cyanide and the mixture was incubated at 50°C for 20 minutes. 2.5 ml of TCA was added to the mixture which was centrifuged at 3000 rpm for 10 minutes. Finally 2.5 ml of supernatant solution was mixed with 2.5 ml distilled water and 0.5 ml ferric chloride. Absorbance was measured at 700 nm in a visible spectrophotometer against blank and compared with standard (Gallic acid) (Oyaizu et al., 1986) absorbance of the reaction mixture indicates stronger reducing power.

In Vivo Anti-Oxidant Study

Experimental Animals:C57BL/6 (30-35 g) of male sex mice (6 animals/group/cage) were purchased from National Institute of Nutrition (Hyderabad, India). The animals were housed in ventilated plastic cages and maintained at 12 hour Light/12Hour Dark Cycle with free access to food and water. All the experiments involving animals were performed according to the standard protocols from National Institute of Nutrition (NIN) guidelines, after getting proper approval.

Cell line: The B16F-10 melanoma cell line was purchased from National Centre for Cell Science (NCCS, Pune, India). The cells were maintained in RPMI 1640 medium buffered with 2 g/L of HEPES and sodium bicarbonate, and supplemented with dextrose, penicillin, streptomycin and 10% of fetal bovine serum. The cells were maintained in a humidified atmosphere containing 5% CO_2 at 37ºC. When needed for experiments (or

during routine passaging steps), the cells were harvested with trypsin: EDTA (0.05: 0.03 [W/V] solution, and then washed in phosphate buffered saline (PBS, pH 7.4). For the animal experiments, the recovered cells were adjusted to 1×10^6 cells/ml in PBS and then 100μl of the suspension was injected into the tail-vein of the mice to develop lung metastasis. (Sheeja K, 2010)

In Vivo acute drug toxicity study: Overnight-fasted C57BL/6 mice of male sex weighing 25-35 g were used and the doses ranging from 10-100 μg were used. Animals were divided into 5 groups of 3 animals each. Each group of animals was given different doses of drug ranging from 10, 25, 50, 75 and 100 μg/kg via i.p., the toxicological effects were observed in terms of mortality and expressed as LD50. The total period of observation was about for 72 h (Drug Evaluation and Research, 1996)

Experimental Design for *in vivo* antioxidant study: **Group 1**: Normal. (n=6). **Group 2**: Cancer alone [1 million cells (10^6 cells) of B16F-10 cell line injected by tail vein] (n=6). **Group 3**: Treated [Cancer+50μg/dose i.p., Essential oil of *Wedelia chinensis (Osbeck)* for 21 days] (n=6).

Sample Collection: At the end of the experimental period (22nd day), the animals were sacrificed with excess of anesthetic agent chloroform. Lung and liver tissues were immediately excised out and placed in the ice cold saline, blotted dry and then weighed. The weighed lung and liver tissues were homogenized in 0.1 M Tris-HCL buffer and then centrifuged at 2500 rpm for 10minutes and then the supernatant obtained was separated and used for the estimation of Glutathione Peroxidase, Catalase, Protein, Reduced glutathione, Nitric oxide and Superoxide Dismutase. The blood was collected by cardiac puncture and then the serum was separated by centrifugation at 2000 rpm for 10minutes and used for the estimation of above mentioned assays.

Assay of Reduced glutathione (GSH) level in Serum, Lung and Liver tissues: Estimation of reduced glutathione was carried out based on the method described by Hafeman et al. (1974). Briefly, 0.5 ml of tissue homogenate/serum was precipitated with 125 μl of 25% TCA and cool in ice for 5 min. The mixture was diluted with 0.6ml of 5% TCA and centrifuged for 10 min at 5000 rpm. 0.3 ml of the supernatant was separated and added with 0.7 ml of phosphate buffer and 2 ml Ellman's reagent. The yellow colour developed was read in a colorimeter at 412 nm. A series of standards (20-100 μg) were treated in a similar manner along with a blank containing 1ml buffer. The amount of GSH was expressed in μg/mg protein for tissue and μg/ml serum.

Nitric oxide (NO) radical scavenging activity in Serum, Lung and Liver tissues: Estimation of nitrite was carried out based on the method described by Stuehr et al. (1989). Briefly, nitrite in the serum and lung, liver tissues was determined by incubating 100 μl sample with equal amount of Griess reagent (one part of 0.1% N (1-naphthyl)-diamine dihydrochloride in distilled water and 1 part 1% sulphanilamide in 5% concentrated H_3PO_4) for 10 minutes at room temperature. Absorbance was measured at 540 nm and the amount of nitrite was calculated from the $NaNO_2$ standard curve. The amount of nitric oxide was expressed in μl/ml for serum and μm/gm tissue for tissue.

Assay of lipid peroxidation using thiobarbituric acid reactive substances (TBARS) in Serum, Lung and Liver tissues: Estimation of lipid peroxidation was carried out based on the method described by Ohkawa et al. (1972). Briefly, to 0.1 ml of tissue homogenate/serum, 0.2 ml of SDS, 1.5 ml of acetic acid solution and 1.5 ml of aqueous solution of thiobarbituric acid (TBA) were added. The mixture was made up to 4.0 ml with distilled water and heated in a water bath at 95°C for 60 minutes. After cooling with tap water, 1.0 ml of distilled water and 5.0 ml of a mixture of n-butanol and pyridine (15:1) were added and shaken vigorously. After centrifugation at 3000 rpm for 15 minutes, the organic layer was removed and read its absorbance at 532 nm. The values are expressed in nmoles/l for serum and nmoles/mg protein for tissue.

Assay of Superoxide Dismutase (SOD) in lung and liver tissues: The assay of enzyme Superoxide Dismutase was carried out by McCord et al. (1969). Briefly, 1 ml of tissue homogenate, 0.25 ml of chloroform and 0.5 ml of ethanol were added and mixed vigorously with vortex mixer. This mixture was centrifuged at 1800 rpm for 6mins. 100 μl of supernatant was taken and transferred into a test tube. The volume was made up to 2.25 ml with phosphate buffer (pH 7.8). Then 0.2 ml of EDTA/ Na CN, 0.1 ml NBT (nitro blue tetrazolium), and 0.5 ml riboflavin were added in the mixture and the reading were taken at 560 nm. After taking the initial reading the tubes were kept for illumination inside the chamber for 15 min. After 15 min the tubes were taken out and again taken the OD value at 560 nm. The difference between initial and final reading was recorded. The specific activity of the enzyme was expressed as Units/mg protein.

Assay of Catalase (CAT) in Serum, Lung and Liver tissues: Estimation of Catalase was carried out based on the method described by Sinha et al. (1972). Briefly, to 0.9 ml phosphate buffer, 0.1 ml tissue homogenate/serum and 0.4 ml H_2O_2 were added. The reaction was arrested after 60 sec by adding 2.0 ml dichromate-acetic acid mixture (ratio 1:4). The tubes were kept in a boiling water bath for 10mins, cooled and the colour developed was read at 590 nm. Standards in the concentration range of 20-100 μM were used. The specific activity of the enzyme was expressed as Units/ml for serum and Units/mg protein for tissue.

Assay of Glutathione Peroxidase (GPx) in Serum, Lung and Liver tissues: Estimation of Glutathione Peroxidase was carried out based on the method described by Rotruck et al. (1973). Briefly, to 0.2 ml of Tris-HCL buffer, 0.2 ml EDTA, 0.1 ml sodium azide and 0.2 ml tissue homogenate/serum were added and mixed well. To this 0.2 ml reduced GSH followed by 0.1 ml of H_2O_2 were added. The contents were mixed well and incubated at 37°C for 10mins along with a tube blank containing all reagents except the sample. After 10 mins, reaction was arrested by the addition of 0.5 ml of 10% TCA. The tubes were centrifuged and the supernatant was assayed for GSH utilization by colorimetric reading at 340 nm. The enzyme activity was expressed in Units/ml for serum and Units/mg protein for tissue.

Histopathological analysis of lung: Lung tissues (Tumor nodules) were fixed in 10% formaldehyde, dehydrated and embedded in paraffin wax. 4µm sections were then stained with Hematoxylin and Eosin (H & E) and mounted in DPX and examined under a microscope for histopathologic changes of lungs cancer. (Balaji et al., 2011)

Statistical Analysis

Data was statistically analyzed using one-way ANOVA as primary test followed by Dunnett's test using Graph pad InStat 3.0 software. All the results were expressed as mean±S.D for 6 animals in each group. Data are expressed as mean±S.D. Significant at P<0.05*, P<0.01** and P<0.001***.

Results

The total compounds identified in GC-MS analysis were 10 out of these compounds Carvocrol and Trans-Caryophyllene were found to be the major compounds with 96% comparison with the wilily and NBS libraries (Manjamalai et al., 2010).

The free radical scavenging activity of the essential oil was dose dependent and the result obtained was compared with standard antioxidant such as Ascorbic acid and Gallic acid with the different concentration such as 5, 10, 25, 50, 100 µg/ml.

Table 1 show the DPPH free radical scavenging activity of *Wedelia chinensis (Osbeck)* essential oil the standard antioxidant used for comparison was Ascorbic acid. The percentage of inhibition of DPPH free radical were observed as 6.22 (±0.01), 15.14 (±0.02), 33.46 (±0.01), 48.96 (±0.66) and 65.91 (±0.56) whereas for ascorbic acid it was found to be 8.44 (±0.23), 19.04 (±0.06), 32.59 (±0.33), 56.66 (±0.03) and 85.32 (±0.48) respectively. The IC_{50} value calculated for the essential oil was found to be 48 µg/ml whereas for standard ascorbic acid it was found to be 62 µg/ml.

Table 1 shows the percentage inhibition of hydroxyl radical scavenging were observed as 14.02 (±0.29), 23.28 (±0.05), 32.27 (±0.56), 47.20 (±0.02), 68.04 (±0.33) µg/ml whereas for the standard ascorbic acid it was found to be 20.25 (±0.37), 35.20 (±1.17), 49.66 (±0.57), 50.23 (±1.01) and 82.99 (±0.52) respectively. The IC_{50}value calculated for the essential oil was found to be 51µg/ml whereas for standard ascorbic acid it was found to be 62µg/ml.

Table 1 shows the reducing power activity of the essential oil was observed to be having increased absorbance with increase in the concentration of the essential oil and the results obtained were compared with standard gallic acid.

For the analysis of antioxidant activity of *Wedelia chinensis (Osbeck)* essential oil *in vivo* animal models C57BL/6 mice was used and the cell line used was B16F-10 melanoma metastatic cell line. The experiment was performed for 21 days and after the 22nd day the animals were sacrificed under ether anesthesia. Lungs, liver and serum were collected and different antioxidant systems were used for the analysis and the results obtained were summarized below.

Table 2 shows the GSH level in the essential oil treated it was found to be increased (0.135±0.021 -Lung tissue, 0.207**±0.001 Liver tissue and 3.858**±0.011 Serum respectively on 22nd day) and it was statistically significant when compared with cancer induced group and normal group. Whereas, the level of Nitric oxide was found to be increased in the cancer induced group (35.58±0.012 Lung tissue, 42.49±0.393 Liver tissue, 37.32±0.342 Serum) in the essential oil treated group it was noticed that the level of nitric oxide was found to be decreased due the activity of the essential oil (28.27**±0.435 Lung tissue, 33.37**±0.166 Liver tissue, 26.08**±0.401 Serum) respectively on 22nd day of the experiment.

Table 2 shows the essential oil treatment increases the SOD level (3.661**±0.174 Lung tissue, 5.68**±0.179 Liver tissue) whereas in the cancer induced group the level of SOD was found to be decreased and the results obtained were statistically significant when compared with the cancer induced and normal group. It was noted that the lipid peroxidation was found to be increased in the liver tissue of the cancer induced group (0.740±0.08) and in the essential oil treated group it was lipid peroxidation

Table 1. In vitro Antioxidant Activity

Concentration (µg/ml)	% Inhibition
DPPH free radical scavenging activity	
Ascorbic acid (IC_{50}Value- 48 µg/ml)	
5	8.44 (±0.23)
10	19.04 (±0.06)
25	32.59 (±0.33)
50	56.66 (±0.03)
100	85.32 (±0.48)
Wedelia chinensis (Osbeck)(IC_{50}Value- 62 µg/ml)	
5	6.22 (±0.01)
10	15.14 (±0.02)
25	33.46 (±0.01)
50	48.96 (±0.66)
100	65.91 (±0.56)
Hydroxyl radical scavenging activity	
Ascorbic acid (IC_{50}Value- 51 µg/ml)	
5	20.25 (±0.37)
10	35.20 (±1.17)
25	49.66 (±0.57)
50	50.23 (±1.01)
100	82.99 (±0.52)
Wedelia chinensis (Osbeck) (IC_{50}Value- 62 µg/ml)	
5	14.02 (±0.29)
10	23.28 (±0.05)
25	32.27 (±0.56)
50	47.20 (±0.02)
100	68.04 (±0.33)
Reducing power	
Gallic acid	
5	0.348 (±0.003)
10	0.443 (±0.001)
25	0.583 (±0.001)
50	0.726 (±0.002)
100	0.776 (±0.001)
Wedelia chinensis (Osbeck)	
5	0.378 (±0.015)
10	0.372 (±0.005)
25	0.515 (±0.003)
50	0.784 (±0.003)
100	0.828 (±0.001)

Table 2. In vivo Antioxidant Data *Wedelia chinensis (Osbeck)* Essential Oils

Treatment	Reduced Glutathione			Nitric Oxide		
	Lung Tissue	Liver Tissue	Serum	Lung Tissue	Liver Tissue	Serum
Group:1	0.144 ±0.004	0.226 ±0.002	7.5 ±0.194	24.78 ±0.099	26.22 ±0.223	29.37 ±0.398
Group:2	0.116[a] ±0.001	0.164[a] ±0.004	1.86[a] ±0.011	35.58[a] ±0.012	42.49[a] ±0.393	37.32[a] ±0.342
Group:3	0.135[b] ±0.021	0.207[b] ±0.001	3.858[b] ±0.011	28.27[b] ±0.435	33.37[b] ±0.166	26.08[b] ±0.401
	Catalase			Glutathione Peroxidase		
	Lung Tissue	Liver Tissue	Serum	Lung Tissue	Liver Tissue	Serum
Group:1	84.41 ±0.209	87.56 ±0.068	36.27 ±0.009	3.473 ±0.120	7.251 ±0.030	1.043 ±0.022
Group:2	62.85[a] ±0.037	47.62[a] ±0.062	25.62[a] ±0.088	1.845[a] ±0.016	5.743[a] ±0.243	0.65[a] ±0.022
Group:3	73.52[b] ±0.162	68.79[b] ±0.124	32.55[b] ±0.018	2.32[b] ±0.016	6.47[b] ±0.060	0.94[b] ±0.007
	Lipid peroxidation			Superoxide Dismutase		
	Lung Tissue	Liver Tissue	Serum	Lung Tissue	Liver Tissue	
Group:1	0.995 ±0.002	1.036 ±0.001	0.026 ±0.002	4.665 ±0.216	6.408 ±0.124	
Group:2	2.186 ±0.001[a]	1.956 ±0.001[a]	0.057 ±0.001[a]	2.568[a] ±0.1366	5.732[a] ±0.083	
Group:3	1.535[b]±0.003	1.426[b] ±0.002	0.036[b]±0.001	3.661[b] ±0.174	5.684[b] ±0.179	

*Each value is expressed as mean ± S.D. for six mice in each group. GSH unit: μg/mg protein for tissues and μl/ml for serum and NO unit: μg/gm Tissue for tissue and μl/ml for serum. LPO unit: n moles/mg protein for tissues and n moles/L for serum and SOD unit: Units/mg protein for tissue. Catalase unit: Units/mg protein for tissues and Units/ml for serum and Glutathione peroxide unit: Units/mg protein for tissue and Units/ml for serum. [a]Statistically compared Treated with Cancer, [b]Statistically compared Treated with Normal, **P<0.01

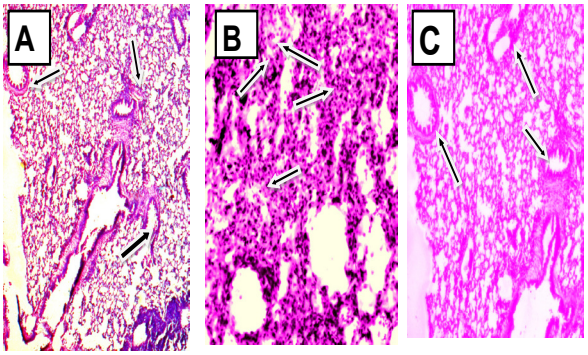

Figure 1. Histopathological Analysis of Lung (40X). A) Normal; B) Cancer control with massive growth around the bronchioles and infiltration of melanoma in the interstitium of the lung; C) Essential oil Treated with significant reduction in tumor mass and regeneration of alveolar passage with ciliated columnar epithelial cells

was found to be decreased in liver tissue (0.623**±0.02) respectively which was statistically significant when compared with the cancer induced and control group on 22nd day.

Table 2 shows that the essential oil of *Wedelia chinensis (Osbeck)* increases both the level of Catalase and Glutathione peoxidase in the lung and liver tissues whereas in the serum the level of Catalase was found to be decreased on the 22nd day (2.32**±0.016 Lung tissue 6.47**±0.060 Liver tissue, **0.94±0.007 Serum) on the other hand the level of the level of GPx in the liver the range was found to be decreased in the essential oil treated group when the result was compared with the cancer induce group and control group whereas the level of GPx in the lung tissue was found to be low (76.2±1.66) and this was not found to be statistically significant when compared with the untreated group and control respectively.

The histopathological study using hematoxylin and eosin stained sections of lung tissues are shown in Figure 1. The lung from healthy normal animal shows normal architecture of lungs with bronchioles, alveoli and interstitium (Figure A) The lungs of positive control (Cancer alone) animals showed massive tumor growth around the bronchioles and infiltration of metastatic colonies of melanoma in the interstitium of the lung. Increased fibrosis reduces alveolar space, which leads to reduction in vital capacity of the lung (Figure B). Simultaneous administration of essential oil of *Wedelia chinensis (Osbeck)* at 50 μg/ml/animal showed significant reduction in tumor mass and regeneration of alveolar passage with ciliated columnar epithelial cells. Lungs of the essential oil treated animals were almost similar to the healthy normal lung (Figure C).

Discussion

The work was performed to determine whether the essential oil of *Wedelia chinensis (Osbeck)* is capable of reducing oxidative stress due to cancer development using *in vivo* animal models. DPPH free radical scavenging assay was performed on the basis of reactivity of test compounds with a stable free radical. Due to the presence of odd electron, 2, 2-Diphenyl-Picryl Hydrazyl radical (DPPH) produces a strong absorption band at 517 nm in visible spectroscopy (Alluri et al., 2009)

Antioxidant efficiency is often associated with their ability to scavenge stable free radicals. In the present study, the DPPH scavenging activity of the samples was compared with reference compound, ascorbic acid. The IC_{50} value obtained for the essential oil of *Wedelia chinensis (Osbeck)* shows that the essential oil of *Wedelia chinensis (Osbeck)* (48 μg/ml) poses a moderate antioxidant activity when compared to the reference antioxidant ascorbic acid used which showed (62 μg/ml) a very potent antioxidant activity.

In general, out of all the reactive species (ROS),

the hydroxyl (OH^-) ROS is found to be one of the most reactive and physiologically harmful free radical, and this was suspected in pathologies such as atherosclerosis and oncogenesis etc.., In this study, the 50% inhibition concentration of the essential oil for scavenging the hydroxyl activity was found at 51 μg/ml whereas that of for the standard antioxidant it's at 62 μg/ml. This clearly depict that the essential oil of *Wedelia chinensis (Osbeck)* pocesses the capability to scavenge the hydroxyl radical produced even though the activity is somewhat moderate when compared with the ascorbic acid which has shown strong antioxidant activity. Herbal based drugs containing free radical scavengers are now gaining importance in preventing and treating several diseases and disorders (Dharmendra et al., 2009).

Phenolics and flavonoids are the major constituents noted in most of the plants and it has been reported by many researchers that they pocesses antioxidant and free radical scavenging activity (Yerra et al., 2005). The reducing power activity of essential oil of *Wedelia chinensis (Osbeck)* was compared with the standard Gallic acid at the same concentration used for the essential oil. The increase in the essential oil concentration eventually increases the absorbance and this reducing power activity was found to be high when compared with the standard Gallic acid. The absorbance observed for essential oil of *Wedelia chinensis (Osbeck)* at 50μg/ml was 0.776 and for 100 μg/ml it was 0.776 but for Gallic acid at 50 μg/ml it was 0.784 and for 100 μg/ml it was 0.828 respectively.

Over production of free radicals results in oxidative stress, which leads to damage to macro molecules (Ebubekir et al., 2007). Glutathione, a potent inhibitor of the neoplastic process, plays an important role in the endogenous antioxidant system. High concentration of glutathione is found in liver and hence it is known to have a key function in the protective process. In our study, the GSH level in the treated mice was found to be significantly high in liver and lung tissues and even in serum whereas, in the cancer induced control mice it was found to be lower and this clearly indicates that the essential of *Wedelia chinensis (Osbeck)* have potent antioxidant activity. Nitric oxide is a multi function free radical species that is implicated in a variety of physiological and pathological processes. Various studies have showed that nitric oxide synthesis was high in tumor tissue and in plasma (Robert et al., 2007). Research in the past few years clearly implicate an important role for nitric oxide in the development of many human cancers and this may be due to the fact that NO is an important signaling molecule and has the capacity to alter many cellular processes depending upon the rate of production. The molecular mechanism of NO and its role in cancer is still under research and in our study the level of NO was found to be decreasing in the treated group which shows that the drug has the capability to induce apoptosis and further leads to the reduction of cancer in the animals.

Lipid peroxidation is well-established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress in cells and tissues. These lipid peroxides are unstable and decompose to form a complex series of compounds like reactive carbonyl compounds. Measurement of malondialdehyde [MDA] has been used as an indicator of lipid peroxidation (Beer et al., 2008). In the present study, the level of MDA released was found to be high in the cancer induced group and this is because of tissue damage during the development of cancer whereas in the treated group the level of MDA released in the tissue and serum was found to be low because, of the scavenging activity of essential oil. Glutathione Peroxidase (GPx) is an enzyme family with Peroxidase activity. The major biological role is to protect the organism from oxidative damage and reduce lipid hydro peroxides to their corresponding alcohols and to reduce hydrogen peroxide to water. GPx 4 depletion causes lethal effect in knockout mice during embryonic development. We have observed an elevated GPx activity upon treatment with essential oil of *Wedelia chinensis (Osbeck)* when compared to untreated cancer control.

It has been already reported that the Superoxide dismutase (SOD) plays an important role in the biological defense mechanism through dismutation of endogenous cytotoxicity superoxide radicals to H_2O_2 (Chelikani et al., 2004). In general, Superoxide dismutase catalyses the Superoxide into H_2O_2 which has been eliminated by glutathione peroxidase or Catalase. In our study, the administration of essential oil significantly increases the SOD and Catalase levels in a dose dependent manner. Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen (Sumner et al., 2006). Moreover, one Catalase molecule can convert millions of molecules of H_2O_2 to H_2O and O_2 each second (Gaetani et al., 1996).

In conclusion, to conclude the study, it is found clearly that the essential oil of *Wedelia chinensis (Osbeck)* showed a significant and potent antioxidant activity both *in vitro* and *in vivo* conditions by scavenging free radicals and by enhancing the level of endogenous antioxidants in experimental mice. Hence the use of essential oil of *Wedelia chinensis (Osbeck)* will be a cheaper and natural drug, to replace the commercially available chemical drug and also without any side effects.

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