Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 31s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Evaluation of the Comparative Genoprotective Effect of Daucus carota and Aloe vera

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Cite this paper as: Deepak Yadav, Manju Prajapati, (2025) Evaluation of the Comparative Genoprotective Effect of Daucus carota and Aloe vera. *Journal of Neonatal Surgery*, 14 (31s), 580-587.

ABSTRACT

Cancer is a deadly disease with a high incidence rate, with breast, ovarian, prostate, and lung cancer being the main types in women and men. In India, breast cancer cases are increasing among wealthy women. Chemotherapy remains the main treatment, but effective therapy depends on early diagnosis and progression stages. Plants have long been used as a source of medicines, with traditional medicine systems like Ayurveda, Unani, and Siddha using plant extracts for various cancer treatments. Natural products from medicinal plants have been discovered to be effective in cancer therapy, with 14 of the top 35 drugs in 2000 worldwide. Further studies could isolate bioactive compounds, reduce chemotherapy side effects, conduct clinical trials, pharmacokinetic studies, and conduct surveillance and post-marketing surveillance. Aloe barbadensis, a perennial, drought-resistant plant, is known as the healing plant due to its wound and burn healing properties. It has been used in health, beauty, medicine, and skin care for centuries. The pharmacologically active ingredients in aloe are concentrated in inner parenchymatous tissue called aloe gel and outer pericyclic tubules called aloe sap or aloe juice. These bioactive compounds are effective in treating various conditions, including burns, allergic reactions, rheumatoid arthritis, rheumatic fever, acid indigestion, ulcers, diabetes, skin diseases, dysentery, diarrhea, piles, and digestive system inflammatory conditions. Aloe vera is also used in cosmetic products to provide a healthy, supple skin look, reduce wrinkles, cure acne, rejuvenate, and give it a youthful glow. Dacus carota, also known as carrot, is a plant in the Apiaceae or Umbelliferae family, classified into twelve subspecies. The wild carrot was traditionally used for medicinal purposes by ancient Greeks and Romans, with its antioxidant, anticancer, anti-inflammatory, gastroprotective, hepatoprotective, antibacterial, and antifungal activities confirmed in-vitro and in-vivo over the past two decades. The study aims to assess the Genoprotective effect of Daucus carota and Aloe vera using Invitro and Invivo models for their toxicity analysis. The results suggest that the Genoprotective effect of the plan exctracts showed a concentration dependant potency and leads a way for a herbal approach for a successful Genoprotective agent.

Keywords: *Methanolic extract; Daucus carota and Aloe vera; Genoprotective effect; Cancer.*

1. INTRODUCTION

Aloe barbadensis, a perennial, drought-resistant plant, is known as the healing plant or "silent healer" due to its wound and burn healing properties. It has been used for centuries in health, beauty, medicine, and skin care, with a significant role in indigenous systems like ayurveda, siddha, unani, and homoeopathy. The pharmacologically active ingredients in aloe are concentrated in inner parenchymatous tissue called aloe gel and outer pericyclic tubules called aloe sap or aloe juice (1-3). These bioactive compounds are effective in treating various conditions, including burns, allergic reactions, rheumatoid arthritis, rheumatic fever, acid indigestion, ulcers, diabetes, skin diseases, dysentery, diarrhea, piles, and digestive system inflammatory conditions. The polysaccharides in the gel of the leaf contribute to its health benefits (4). *Aloe vera* is also used in cosmetic products to provide a healthy, supple skin look, reduce wrinkles, cure acne, rejuvenate, and give it a youthful glow (5).

Daucus carota, also known as carrot, is a plant in the Apiaceae or Umbelliferae family, classified into twelve subspecies. The most well-known are D. carota ssp. sativus and D. carota ssp. boissieri (red carrot). The plant has been selectively bred into a cultivated form, D. carota ssp. Sativus (6, 7). The chemical composition of different subspecies reveals the presence of terpenes, phenolics, and flavonoids. The plant extracts also differ between subspecies and the same subspecies. The wild carrot was traditionally used for medicinal purposes by ancient Greeks and Romans, with its antioxidant, anticancer, anti-

inflammatory, gastroprotective, hepatoprotective, antibacterial, and antifungal activities confirmed in-vitro and in-vivo over the past two decades (8-10). The study aims to assess the Genoprotective effect of *Daucus carota and Aloe vera* using Invitro and Invivo models for their toxicity analysis.

2. MATERIALS AND METHODS

2.1 Processing and Preparation of the methanolic extracts of *Daucus carota* and *Aloe vera* (MEDC and MEAV)

The fruits of *Daucus carota* and leaves of *Aloe vera* were shade dried at room temperature. The plant parts were grinded into coarse powder and used for further investigations. 125 g of coarse fruits powder was subjected to defatting for the removal of wax and lipids. The weighed powder was packed in the thimble and were extracted using methanol using Soxhlet's device. It was then refluxed with 600ml of petroleum ether 60-80° till complete removal of fat material. The defatted marc was collected from the thimble and was soaked in 400-500ml of purified water. These containers were kept in cool and dark place with periodic stirring for 48 hrs. After 48 hrs, solution was filtered through Whatman filter paper no.1. The filtrate was dried in a rotary evaporator and the obtained dried residue was used as crude extract for further investigations. The abbreviation used for methanolic extracts of *Daucus carota* and *Aloe vera* (MEDC and MEAV), respectively.

2.2 Acute Toxicity Study

Total 6 rats of 10-12 weeks age were selected and randomly divided into 2 groups. Group I was vehicle control group which received vehicle (gum acacia 1% w/v in distilled water) while group II was test group that received methanolic extracts of *Daucus carota* and *Aloe vera* (MEDC and MEAV). Each group consisted of 3 animals (females). Females were nulliparous and non-pregnant (11).

2.3 Evaluation of Genoprotective Effect of methanolic extracts of *Daucus carota* and *Aloe vera* (MEDC and MEAV)

2.3.1 In vitro assay

The study focuses on the detection of DNA fragmentation (12), comet assay (13), and micronuclei formation (14) in tissue culture dishes.

2.3.2 In vivo assay

Seventy adult male mice weighing 28–30 g were used in the current study. Standard food pellets and tap water were supplied ad libitum. Food pellets were obtained from the Ashirwad Brand, Chandigarh. Animals were randomly allocated into 7 groups (70 rats each). Mice of the 1st group served as normal control group. Group 2 received tumour inoculation with no treatment and served as tumour inoculation-control group (TIC). Group 3 received tumour inoculation and standard drug Cisplatin (3.5 mg/kg, ip, on 1st day only). Groups 4 and 5 received tumour inoculation and MEDC (150 and 300 mg/kg/day, P.O.) respectively for 13 days. Groups 6 and 7 received tumour inoculation and MEAV (150 and 300 mg/kg/day, P.O.) respectively for 13 days. All the animal was weighed on the day of tumour inoculation and then for every three days. Treatment was given on 3rd, 5th, 7th, 9th, 11th, and 13th day of tumour inoculation p.o. (15).

2.3.3 Parameters to be monitored (16-20)

2.3.3.1 Change in body weight as compared to day "0" weight.

Animals were weighed on the day of tumour inoculation and after once in 2 days of the post inoculation period, the % increase in body weight was calculated as follows and compared with normal and TIC controls.

2.3.3.2 Haematological parameters

In order to detect the influence of selected fractions on the haematological status of TIC bearing mice, the parameters were monitored such as White blood cell total count, Red blood cell total count, Haemoglobin content Haematological Parameters were estimated by using automated veterinary blood cell counter.

2.3.3.3 Tumour volume

On 14th day of the tumour inoculation, the animals were sacrificed and the peritoneal ascitic fluid was collected into a measuring cylinder and the volume of the ascitic fluid was measured and compared in all the treated and TIC control groups. TIC count was determined using trypan dye exclusion assay.

2.3.3.4 Evaluation of Oxidative Stress Parameters

The piece of stomach isolated above was washed thoroughly with ice-cold 0.1 M phosphate buffered saline (pH 7.4). It was blotted dry and homogenized in 1.15% KCl to prepare a 10% w/v suspension. The suspension was centrifuged at 16000×g for 1 h in a cooling centrifuge at 0 °C. The supernatant was then employed for assessment of lipid peroxidation (LPO), catalase (CAT) and superoxide dismutase (SOD) activity, and reduced glutathione (GSH) content.

2.4 Statistical analysis

All the data would be finally analyzed by appropriate statistical tests and test of significance, multiple comparisons, etc. Data was analysed statistically using ANOVA. Two ways ANOVA was applied followed by Bonferroni's post-test for comparison between treated and untreated plants. One way ANOVA was applied to determine the significance of results between different treatments and Tukey's multiple comparison tests were perform at significance level in different treatments.

3. RESULTS AND DISCUSSION

3.1 Acute Toxicity Study

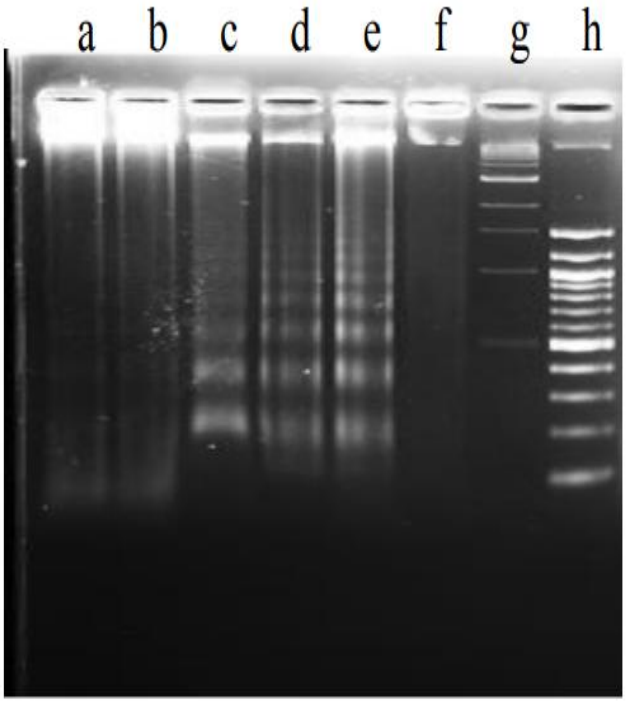
The test drug, MEDC and MEAV, was found to be safe up to a dose of 2000 mg/kg body weight, as observed during toxicity studies. The drug did not cause drug-related toxicity, mortality, abnormal clinical signs, remarkable body weight, or gross pathological changes in animals. The test substance is classified as "unclassified" or "category - 5" according to the Globally Harmonised method.

3.2 Genoprotective Effect of methanolic extracts of *Daucus carota* and *Aloe vera* (MEDC and MEAV) on mice

3.2.1 In Vitro Assay

3.2.1.1 DNA fragmentation Assay

A study on cancer cell lines, MEDC and MEAV, found that methanolic extracts of *Daucus carota* and *Aloe vera* can induce apoptosis. The cells were treated with these extracts for 48 hours, and DNA fragmentation was analysed. The results showed a ladder pattern in HeLa cells treated with MTX, indicating apoptotic cell death, unlike untreated and vehicle control cells. This suggests that these extracts can induce apoptosis (Figure 1).



a. Control
b. Vehicle Control
c. Standard (MTX)
d. MEDC (low dose)
e. MEDC (high dose)
f. MEAV (low dose)
g. MEAV (high dose)
h. DNA ladder marker (100kb)

Figure 1: Effect of selected fractions on DNA fragmentation in HeLa cell lines

3.2.1.2 Single cell gel electrophoresis (COMET) assay for apoptosis

In order to study detailed apoptosis and DNA fragmentation, all the selected cell lines were treated with methanolic extracts of *Daucus carota* and *Aloe vera* (MEDC and MEAV) for 48 hours. Pattern of comet formation shown in Figure 2. Treatment with methanolic extracts of *Daucus carota* and *Aloe vera* (MEDC and MEAV) show significant increase in COMET parameters as compared to untreated cells. Anticancer drug, methotrexate was used as positive control throughout the study which show increase in COMET parameters mentioned above.

3.2.1.3 Micronuclei Formation Assay

Formation of micronuclei is a hallmark of property of drug induced genotoxicity. In order to evaluate the genotoxicity, selected panel of cancer cell lines were treated with methanolic extracts of *Daucus carota* and *Aloe vera* (MEDC and MEAV) for 48 hours. At the end of treatment, cells are harvested and were analysed microscopically for presence of micronuclei especially, bi- tri or multinucleated interphase cells (Figure 3).

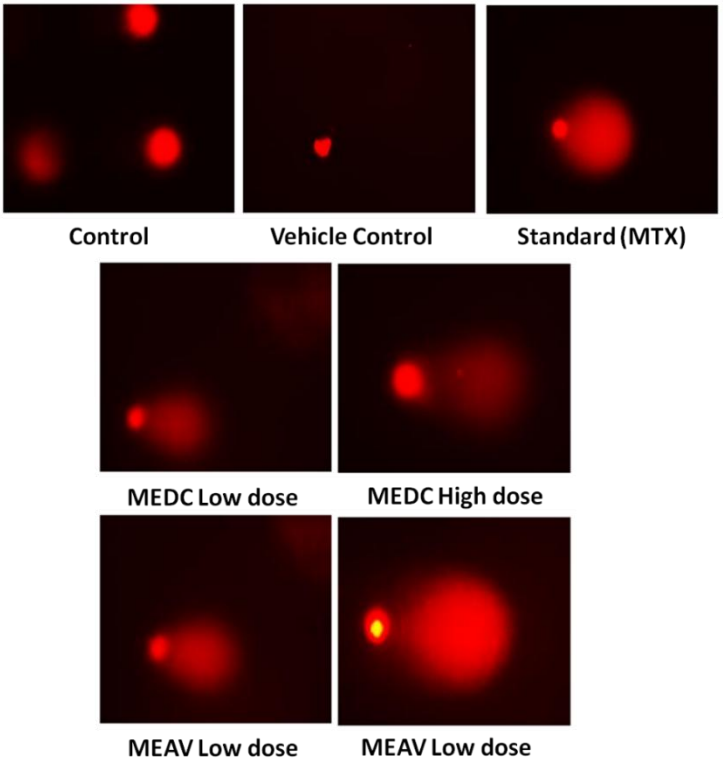


Figure 2: Induction of DNA damage assessed by alkaline comet assay (% Tail DNA and olive tail moment) in HeLa cells treated with different concentrations methanolic extracts of *Daucus carota* and *Aloe vera* (MEDC and MEAV) for 24 hrs

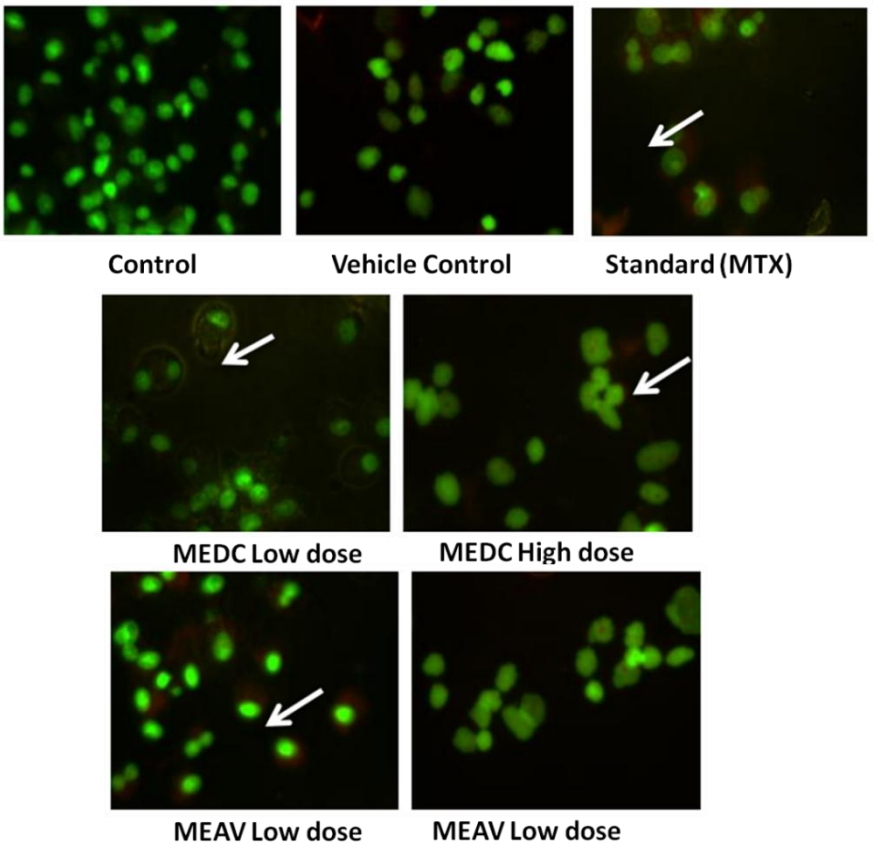


Figure 3: Induction of micronuclei after treatment with selected fractions for 48 hrs in HeLa cell lines

3.2.2 In vivo assay

3.2.2.1 Effect of treatment on change in body weight in EAC inoculated mice

The EAC inoculated mice were found to gain body weight progressively. The maximum gain in tumour weight (39.13%) was observed on day 13th of tumour inoculation. The standard drug, Cisplatin administered on day 1st significantly ($p< 0.05$) reduced the elevated body weight as compared to control from 7th day onwards. MEDC and MEAV (300 mg/kg) showed significant ($p<0.05$) reduction in elevated body weight as compared to EAC control from 7th day onward. MEDC and MEAV (150 mg/kg) showed significant ($p<0.001$) reduction in bodyweight from 9th day onward (Figure 4).

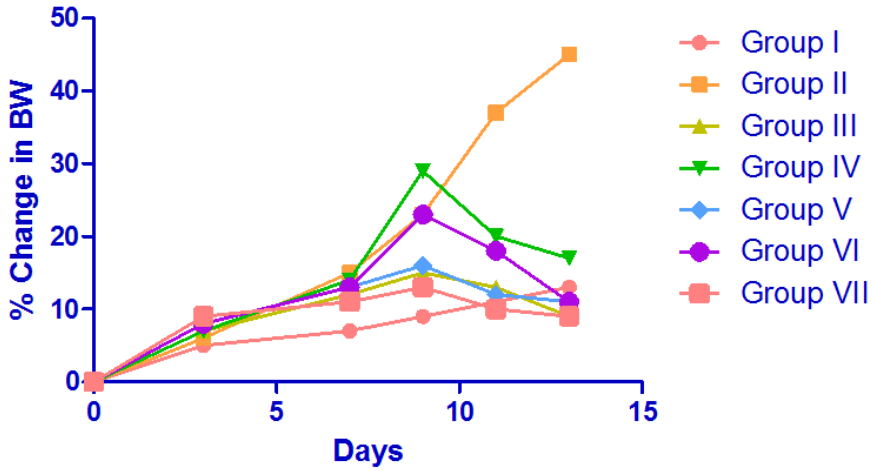


Figure 4: Effect of treatment on change in body weight in EAC inoculated mice (Group 1 trated as Normal Control group; Group 2 received tumour inoculation with no treatment and served as tumour inoculation-control group (TIC); Group 3 received tumour inoculation and standard drug Cisplatin (3.5 mg/kg, ip, on 1st day only); Groups 4 and 5 received tumour inoculation and MEDC (150 and 300 mg/kg/day, P.O.) respectively for 13 days and Groups 6 and 7 received tumour inoculation and MEAV (150 and 300 mg/kg/day, P.O.) respectively for 13 days.)

3.2.2.2 Effect of treatment on Haematological parameters in EAC inoculated mice

The study found that EAC inoculated mice had an increase in WBC count twofold compared to the normal control. Cisplatin administration reversed this increase, while fractions treated mice had a decrease. MEDC and MEAV (300 mg/kg) were more effective in reducing elevated WBC count. RBC count was also significantly reduced in EAC inoculated mice, with MEDC and MEAV improving it. Haemoglobin content was also reduced, but Cisplatin treatment restored it to normal levels (Table 1).

Table 1: Effect of treatment on Haematological parameters in EAC inoculated mice

Group	Group Name	Haematological parameters		
		Haemoglobin	RBC	WBC
G-I	Normal Control	12.20 ± 0.40	9.75 ± 0.40	11.60 ± 0.44
G-II	Tumour Control	7.20 ± 1.15	4.72 ± 0.66	25.48 ± 2.75
G-III	Standard (Cisplatin)	11.47 ± 0.63	9.03 ± 0.59	14.60 ± 0.84
G-IV	MEDC-I	9.76 ± 0.26	8.60 ± 0.48	18.90 ± 3.35
G-V	MEDC-II	10.46 ± 0.64	9.00 ± 0.23	19.34 ± 2.92
G-VI	MEAV-I	8.03 ± 0.33	7.13 ± 0.86	18.36 ± 1.39
G-VII	MEAV-II	9.43 ± 0.96	8.01 ± 0.14	19.86 ± 3.10

3.2.2.3 Effect of treatment on ascitic fluid (Tumour) volume in EAC inoculated mice

The study found that Cisplatin treatment significantly reduced the ascitic cell count and EAC count to 0.59 million cells/ml, with all selected fractions showing a significant reduction in ascitic fluid volume compared to the EAC control, with a reduction of 4.49 ml up to 1.70 ml (Figure 5).

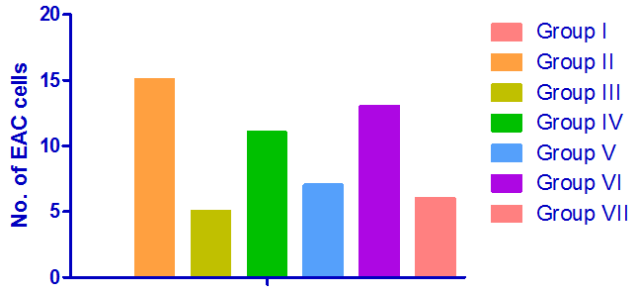


Figure 5: Effect of treatment on Ascitic (EAC) (x106/ml) cell count

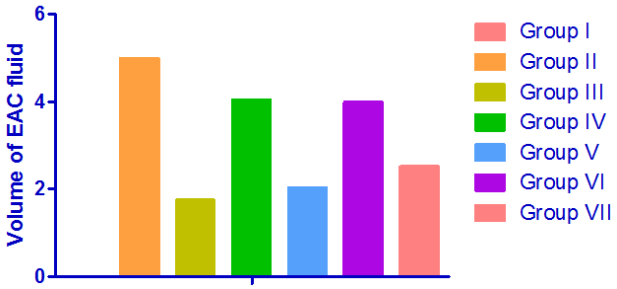


Figure 6: Effect of treatment on Ascitic fluid volume (mL)

(Group 1 trated as Normal Control group; Group 2 received tumour inoculation with no treatment and served as tumour inoculation-control group (TIC); Group 3 received tumour inoculation and standard drug Cisplatin (3.5 mg/kg, ip, on 1st day only); Groups 4 and 5 received tumour inoculation and MEDC (150 and 300 mg/kg/day, P.O.) respectively for 13 days and Groups 6 and 7 received tumour inoculation and MEAV (150 and 300 mg/kg/day, P.O.) respectively for 13 days.)

3.2.2.4 Estimation of Biochemical Parameters

The study found that EAC inoculated mice showed significant differences in reduced GSH content, reduced glutathione content, and Catalase levels compared to the sham control. Cisplatin significantly increased reduced glutathione content, while MEDC and MEAV significantly reduced lipid peroxidation. Additionally, EAC inoculated mice showed a significant reduction in SOD level, with treatment with Cisplatin, MEDC, and MEAV showing significant improvement. These findings suggest that EAC treatment can potentially improve overall health (Figure 7).

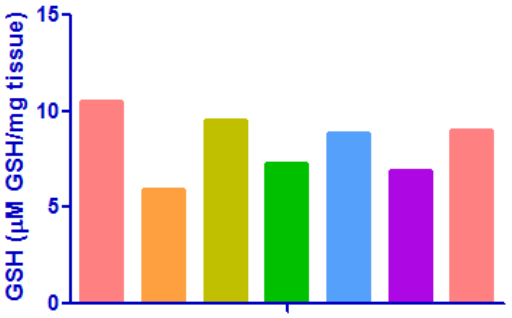


Figure 7a

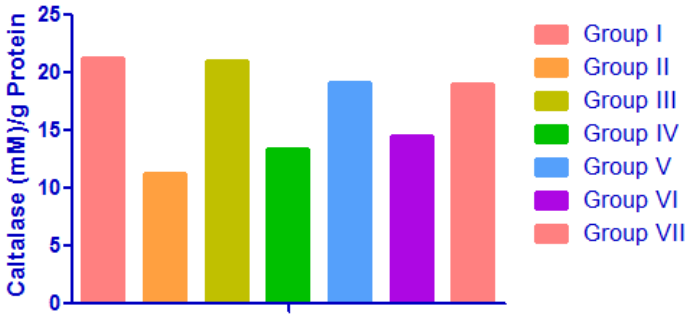


Figure 7b

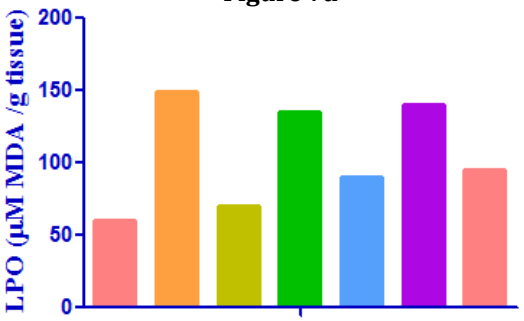


Figure 7c

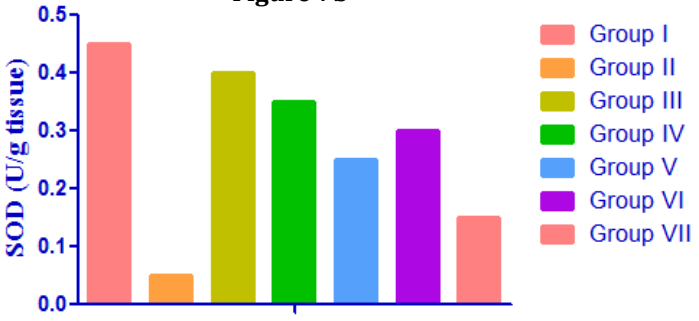


Figure 7d

Figure 7: Estimation of Biochemical Parameters: a) GSH; b) Catalase; c) LPO and d) SOD

4. CONCLUSIONS

Cancer is a lethal disease with a high incidence rate, with breast cancer and ovarian cancer being the main types in women and prostate cancer and lung cancer in men worldwide. In India, breast cancer cases are growing among wealthy women in the city. Chemotherapy remains a major modality for cancer treatment, but an effective therapy depends on early diagnosis and progression stages. Metastatic cancer is often resistant to anti-cancer or cytotoxic drugs and relapses after some time even after chemotherapy. Plants have been an excellent source of medicines for a long time, with traditional medicine systems such as Ayurveda, Unani, and Siddha using plant extracts as potential treatments for various types of cancer. Some of the most important anti-cancer or cytotoxic compounds obtained from plants include Cathranthus roseus, Homoherringtonine, Rhein, Mistletoe, and Betulinic Acid.There is considerable scientific and marketable attention in the ongoing discovery of new anticancer or cytotoxic drugs from plant sources. Natural products discovered from medicinal plants have an important role in the therapy of cancer, with natural sources or their derivatives of natural products including 14 of the top 35 drugs in 2000 based on sales worldwide. Selected plants have a lot of activities for treating different diseases, with the main purpose of the use being to improve health quality and prevent diseases. The selected plants contain various active phytochemical constituents like alkaloids, flavanoids, terpenoids, steroids, glycosides, saponins, tannin, carbohydrates, protein, and gum/mucilage. Further studies could be conducted to isolate these bioactive compounds, reduce chemotherapy side effects, conduct clinical trials, conduct pharmacokinetic studies, and conduct surveillance and post-marketing surveillance.

5. CONFLICT OF INTEREST

None

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