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Zingerone prevents alterations in hematological parameters in DMBA induced mammary cancer rats

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Abstract - Zingerone is present in ginger in a significant amounts and comprises approximately around 9.25%It has a basic phenolic ring attached to the benzene ring with a methoxy group. Zingerone has important beneficial pharmacological properties which offers enormous scope in the field of medicinal chemistry. Zingerone [4-(3-methoxy-4-hydroxyphenyl)-butan-2-one] is known to possess potent antitumor activities. In our study, zingerone was supplemented to rats treated with the carcinogen DMBA, which showed marked protective effects. Blood parameters such as Hb, RBC, WBC, PLT's, PCV, MCHC and MCV were significantly altered on DMBA administrations which were significantly improved on supplementation with zingerone as compared to the untreated control rats.

Key Words: Zingerone, Hb, RBC, WBC, PCV, MCHC and MCV

1.INTRODUCTION

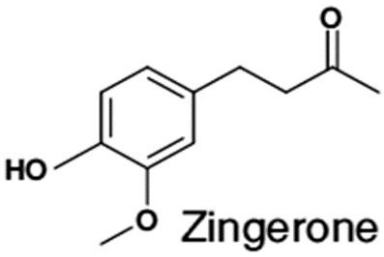

Fig: 1 Structure of zingerone

Cancer is an abysmal ailment and is the leading cause of death in many parts of the world. Cancer may disturb people at all eternities, even fetuses, but the risk varies with age. Cancer has three main methods of treatment: chemotherapy, surgery, and radiation therapy. In recent years, chemotherapy is an important therapeutic method. So designing and synthesizing a new anticancer drug with high efficacy is a an interestiy study area today (1). Animal models are used for developing new methods and approaches to cure and improve human diseases, disability, and other biological processes. (2,3,4). The use of laboratory rats (*Rattus norvegicus*) in research has increased steadily as they are preferred because of their short life cycle, low cost, easy maintenance in limited space (5) and availability of a large database of their characteristics that are useful in interpreting relevant animal data for humans. (6) Thus the rat model is being used extensively in physiology, transplantation, immunogenetics and cancer research, (7) and remains the dominant model for the preliminary testing of all forms of therapeutic and chemical toxicities. (4,8) In this area of research, abnormal treatment-related values could represent changes pertaining to the effects of the treatments such as the toxicity effects, which could be detected by alterations in a series of in vivo analysed parameters. (9) Among these parameters are haematological data which are of great importance in determining such effects. This is because blood plays a major role in the body's transport system and excretion of substances of almost all the body's metabolic processes, and any deviations from normal are detectable in the blood profile. (3) One way of evaluating the haematologic profile is by evaluating the Full Blood Count (FBC) otherwise called Complete Blood Count (CBC). Flow cytometric analysis of reticulocytes is consequently a hypothetically useful tool to spot changes in erythropoiesis. (10) It yields information about production of all blood cells, the oxygen-carrying capacity through RBC indices, haemoglobin and haematocrit. It also provides information about the immune system through the evaluation of the WBC counts especially along with differential counts. CBC is one of the most frequently requested tests in clinical medicine with multiple indications such as anaemia, cancer, infection as well as for monitoring the side effects of drugs cause blood dyscrasias. (11 and 12). Of all the rat models, Sprague Dawley (SD) rats is the most widely used research model in all aspects of biomedical research. (13) The docile nature of the rats provides a variety of health profiles that caters to the needs of specific research.

Cancer chemotherapy causes myelo suppression and anemia (14) and Greenfield due to reduction of both red blood cell (RBC) count and thus the hemoglobin percentage. In support of this anticancer study, hematological parameters have also been studied. The present study was carried out to evaluate the effect of zingerone supplementation on blood parameters in dimethyl benz(a)anthrancene (DMBA) induced mammary carcinogenesis. This study therefore presents the haematological profile such as the Hb, WBC, RBC, platelets and other related parameters, which were determined using a haematology blood analyser.

2. Materials and Methods

Zingerone and DMSO (dimethyl sulphoxide), were purchased from Sigma Chemicals Company New Delhi, India. All other chemicals and reagents used were of analytical grade, obtained from

Hi Media Laboratory Ltd Mumbai, India. DMBA (dimethylbenz (a) anthracene), were purchased from Sigma Aldrich Chemicals Pvt. Ltd., Bangalore, India.

Experimental animals and diet

Six to seven week old female Sprague-Dawley rats, weighing 100–120 g, were purchased from National Institute of Nutrition, Hyderabad, India. Animals were maintained in the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University, Tamilnadu, India. This study was approved by the Institutional Animal Ethics Committee for the Control and Supervision of Experimental Animal (CPCSEA approval no: 1177) guidelines. The animals were maintained under controlled conditions of temperature (24 ± 2C), humidity (50 ± 10%) and 12 h light/dark cycle. Feed and water were provided ad libitum.

A total of 36 rats were randomly divided into six groups and each group consisted of 6 animals. Group I animals served as untreated control and were provided food and water ad libitum throughout the investigational period. Group II animals had a single subcutaneous injection of DMBA (25 mg/kg b.w.) near mammary gland, at the end of the first week. Group III–V rats were administered zingerone at three different doses (10, 20 and 40 mg/kg b.w.) by intragastric intubation once a day starting one week before exposure of the carcinogen and were continued till the end of the experimental period. Group VI rats received oral administration of zingerone alone throughout the investigational period. At the end of 16th week, rats were fasted overnight and sacrificed by cervical decapitation. The blood sample was transferred into properly labelled EDTA sample bottles. Anticoagulated blood was used for the dertermination of erythrocyte count. (15).

Full blood count

CBC was determined using an automated analyser, SFRI 3 Part cell counter (France), which counts and sizes blood cells by detecting and measuring changes in electrical resistance when the cells in the conductive liquid passes through a small aperture of the machine and hence, impedes the current and cause a measurable pulse. (16) The pulse height is proportional to the volume of the sensed particle. If constant particle density is assumed, the pulse height is also proportional to the particle mass. (14) and [17] The various parameters that were determined include total WBC, lymphocyte percent (LY%), mononuclear cell per cent (MO%), granulocyte per cent (GR%), RBC number, haemoglobin concentration (Hgb), meancorpuscular volume (MCV), meancorpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and platelet count (PLT).

Statistical analysis: was carried out using IBM SPSS (17) to determine the mean, and standard deviation (SD). Independent sample t-test was used to compare values for the haematological indices. 'P value' less than 0.05 ($p < 0.05$) was considered to show significant statistical difference.

3. RESULTS

Biological evaluation of body weight, survival of rats

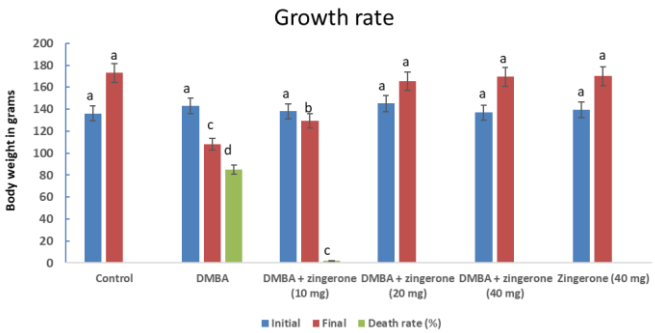

Fig:2 Body Weight and Growth Rate

Values that are not sharing a common superscript in the same column differ significantly at $p<0.05$

The animal's body weight and hematological parameters significantly varied in the normal animals as compared to the control rats. Zingerone supplementation was studied and compared with untreated control and experimentally induced DMBA rats (fig 2). The results of the blood parameters showed significant changes when compared with control (non-treated) rats. The effects of the test compound zingerone on tumor-bearing rats are shown in Figures 3- 6

Effect of zingerone DMBA on RBC, WBC, Hb

Total RBC count, WBC count and Hb levels, showed a significant decrease ($p<0.05$), whereas platelet count and HCT (%) showed a significant increase ($p<0.05$) on exposure to the carcinogen DMBA. In this study, On zingerone (40 mg/kg b.w) feeding to rats exposed to DMBA, mammary cancer rats, the blood paramters were restored to optimal concentrations and were comparable with those of the control rats.

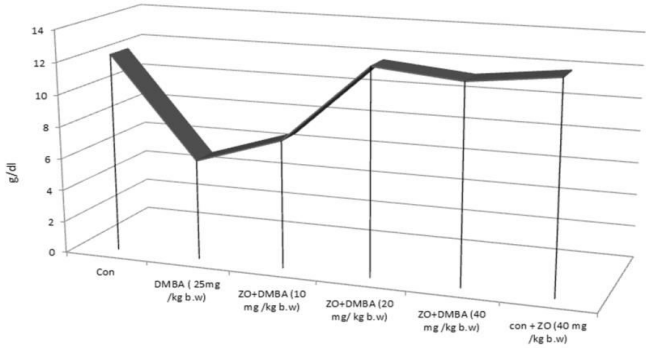

Fig:3 Hb

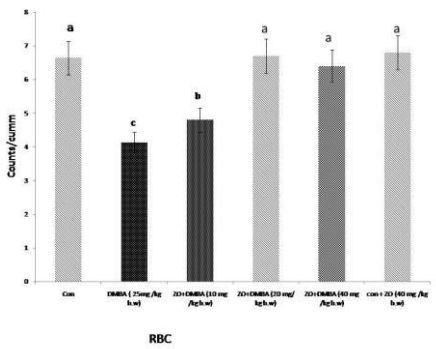

Fig:4 RBC

Figure 3 and 4: Hb and RBC after four months of supplementation with zingerone to DMBA induced rats. Data are presented as the mean ± SD of six rats in each group. Values not sharing a common superscript letter differ significantly at p< 0.05 (DMRT).

Hematological parameters after 4 month of treatment with zingerone to DMBA induced rats

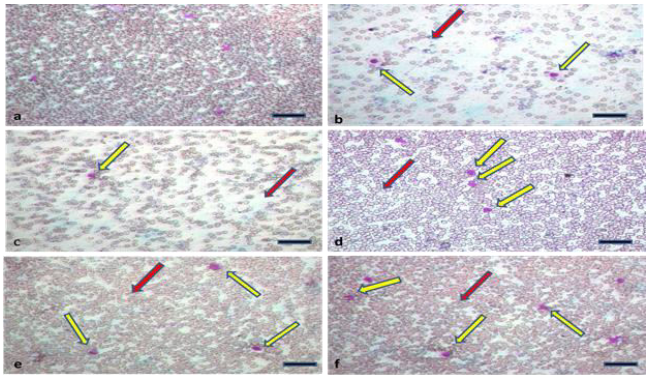

Fig 5 (i) WBC count: Leishman's staining a-control, b-DMBA, c, d, e- DMBA+ zingerone 10,20 and 40 mg kg.b.w respectively, f- control rats+ Zingerone, yellow arrow indicates WBC and red arrow indicates RBC.

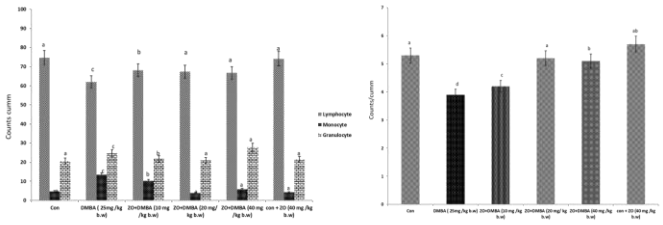

Fig.5(ii) WBC differential count (iii) WBC Total Count

Results are expressed as mean ± standard deviation (S.D.), where n=6. The results were considered statistically significant, when p ≤ 0.05. (DMRT).

Effect of zingerone and DMBA on PLT's, PCV, MCV and MCHC

Figure 6 shows the hematological parameters of the control and experimental rats. Administration of DMBA showed the significant decrease (<0.05) in the levels of PLT's, PCV, MCV and MCHC when compared with the control rats. Supplementation with different doses zingerone significantly showed an increase in the levels of PLT's, PCV, MCV and MCHC. Among the different doses of zingerone supplementation 20 mg/kg b.w showed values near to those of the control rats.

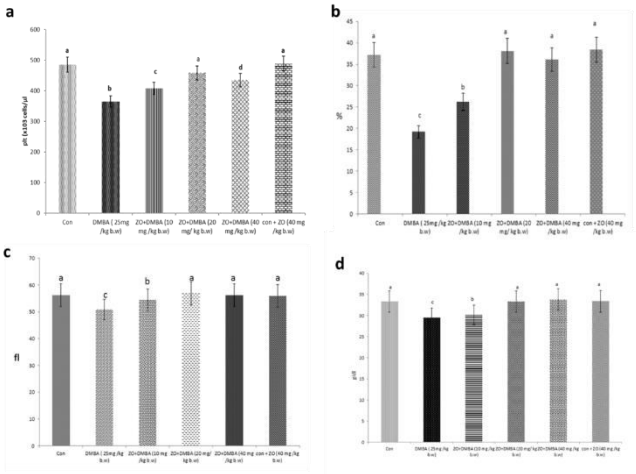

Fig 6: Histological examination of control and treated rats. Fig 6(a) PLT's, fig 6(b) PCV, fig 6(c) MCV, fig 6(d) MCHC. Results are expressed as mean ± standard deviation (S.D.), where n=6. The results were considered statistically significant, when p value ≤ 0.05.

4. DISCUSSION

In this study to confirm the role of zingerone on blood parameters, DMBA was used to induce mammary carcinogenesis. Reliable criteria for judging the value of any anticancer drug (zingerone) is prolonged administration and its effect on of the decrease in WBC levels and increase in hemoglobin [18] (Clarkson et al.,) DMBA showed exposure toxicity to the host during the treatment period, but these parameters were almost restored back to normal values after cessation the treatment.

Hematological parameters are frequently used in routine tests for the diagnosis of many diseases, Systemic inflammatory response facilitates the tumorigenic microenvironment in which immune cells including neutrophils actively participate. Further, it is well documented that cancer related inflammation supports malignant cell proliferation, migration, invasion and metastasis. These systemic inflammatory responses are mostly activated by leukocytes. Many studies have shown that neutrophils, lymphocytes, and platelets among leukocyte subsets are associated with oncologic outcomes and are also used for the prediction of prognosis in several malignancies through routine blood examination. In this study, DMBA (25 mg/kg. b.wt.) was administered to rats and the cancer bearing animals showed decreased lymphocyte count as compared to the control and zingerone alone treated animals. Variation in these hematological parameters may be

due to the increased levels of proinflammatory cytokines such as interleukin -6, interleukin-1 and tumor necrosis factor α.
Hematological parameters were found to be deviated from normal values on treatment with DMBA. Hemoglobin content, RBC counts were found to be decreased and total WBC count were found to be increased on exposure to carcinogen. Decrease in RBC and hemoglobin content are known to result from the hemolyzing power of the compounds [18] (Clarkson et al.,1965). The increase in WBC count can be correlated with an increase in antibody production which helps in survival and recovery of the host. After supplementation with the test compound zingerone, the levels of the hematological parameters were restored almost to near those of the normal values.
Moreover in DMBA induced rats, the tumor weight was found to increase rapidly with time. However, supplementation with the test compound (zingerone) markedly reduced the tumor growth rate. Similar trend was found in inhibiting the cell growth by zingerone.
The lifespan of the DMBA induced rats increased remarkably when treated with the test compound. The prolongation of lifespan of cancer bearing rats is a very important and reliable criterion (19 and 18) for judging the potency of any drug as an anticancer agent. The positive effect of the compound zingerone on DMBA induced cancer bearing rats has further was verified by monitoring blood hematology. Both the RBC and hemoglobin content of cancer bearing rats were found to be decreased gradually with time as found in normal rats. This was probably due to the deficiency of iron in hemolytic or myclopathic condition [20]. Supplementation with zingerone significantly reversed the RBC and hemoglobin contents towards normal. With the growth of tumor, WBC level increased with time. The rise in WBC count in the DMBA induced cancer bearing rats decresed significantly on supplementation with zingerone as compared to the untreated tumor bearing rats. Hematological parameters were parallely studied in normal animals (control rats) to evaluate whether the compound zingerone is toxic to the system. A very slight deterioration in such parameters was observed during the treatment period.
It is well known that systemic inflammation has great impact on tumor progression, Many inflammatory response indicators have been studied to predict tumor response and prognosis after anticancer treatment, such as the levels of lymphocytes, neutrophils, monocytes, platelets, C-reactive protein, erythrocyte sedimentation rate, interleukin-6, and the Glasgow Prognostic Scale which has provided further support for the potency of the compound as an anticancer agent. Zingerone supplementation increased the number of macrophages. This enhancement might produce some cytokines, such as tumor necrosis factor, interleukins, interferon's etc. which in turn may be responsible in destroying tumor cell. [21] Based on the above results, it can be concluded that the test compound zingerone showed pronounced activity as an anticancer agent against DMBA induced Sprague Dawley (SD) rats.

5. CONCLUSION

In conclusion, DMBA- induced mammary carcinogenesis, not only increases the DMBA-induced anemia in Sprague Dawley rats, but also altered the values of all other hematological parameters, which were markedly optimized on zingerone supplementation. Many more investigation has to be carried out with this compound and its derivatives using various cancer cell lines and higher animal models.

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I am Rajagopal Ayyanar doing Ph.D in Annamalai University in the Department of Biochemistry & Biotechnology. I know several technical works and specific field in cancer biology.