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Role of Peper as Bioenhancer in the Treatment of Cancer

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Abstract The current study was undertaken to assess the complementary action of pepper as bioenhancer in the treatment of cancer along with mercaptopurine. Haemoglobin content and RBC count were significantly decreased and total WBC count was significantly increased in the tumour group as compared to the normal group. Treatment with both Mercaptopurine (5mg/kg) and *Piper nigrum* (200mg/kg) alone restored the altered parameters towards the normal values but combination therapy of Mercaptopurine along with *Piper nigrum* showed statistically very significant effects.

Keywords cancer, bioenhancer, pepper, tumour, Haemoglobin, Lipid peroxidation

Introduction

Cancer is a dreadful disease characterized by the irregular proliferation of the cells. As a cell progresses from normal to cancerous, the biological imperative to survive and perpetuate drives fundamental changes in cells behaviour. So, the actual cause of the disease in different sections is still to be explored clearly. Cancer is thus, a class of diseases classified by the type of cell that is initially affected. Today's global scenario indicates that breast cancer and colorectal cancer is the most prominent cancer in case of woman and man. It is estimated that about 80% of human cancers are due to environmental factors, principally chemicals. About 2 to 4% of all cancer deaths are attributed to occupational hazards. A variety of compounds, namely, polycyclic aromatic hydrocarbons, nitrosamines, alkylating agents, and other inorganic and naturally occurring compounds are carcinogenic. Generally, all carcinogens are electrophiles which attack nucleophilic groups in the DNA and RNA and proteins and thus cause genetic damage of the cell. Multidisciplinary scientific investigations are making best efforts to combat this disease but the sure shot, perfect cure is yet to be brought into the world of medicine. To combat cancer United States National Cancer Institute has undergone 2069 anticancer clinical trials, in which over 150 drug combinations have been successfully recorded against cancer [1].

Conventional modalities to treat cancer are surgery, radiotherapy and chemotherapy. Recent approaches have emerged which include drugs inducing differentiation, antimetastatic drugs, antiangiogenic drugs, hypoxic tumour cell specific agents, antitelomerase drugs, biological response modifiers, antisense therapy, gene therapy, radio sensitizers, cancer vaccines, chemoprevention etc. Cancer treatment is usually accompanied by diverse side effects to different body organs. The synthetic chemotherapeutic agents though widely used in treating cancer, suffer from drawbacks of high toxicity (bone marrow suppression, reduced immunity, hepatotoxicity, alopecia, nausea and

vomiting), are costly and not within the reach of a common man. Therefore the challenging task at this moment is to identify the quick and novel methods that can identify and develop molecules, which can be of therapeutic value in cancers. Natural products have long history of use in the service of mankind for the prophylaxis and treatment of several diseases. WHO estimated that 80% of the world population in the developing countries mainly relies on traditional medicines for their health care needs, most of them are plant derived [2].

Herbs have been the highly esteemed source of medicine throughout human history. They are widely used today, is not a throwback to the dark ages but an indication that herbs are a growing part of modern, high-tech medicine. About 25-30 percent of today's prescription drugs contain chemicals derived from plants. Unlike synthetic substances the natural drugs do not give symptomatic relief rather provide complete cure for many diseases. Due to these salient features the importance of herbal drugs has been realized seriously and they have become a preferred way of therapy throughout the globe. World Health Organization has recognized the potential of traditional and folk medicines in the management and self-reliance of health care system and currently it is encouraging and promoting the traditional systems in “National Health Care Programmes” of various countries [3].

Herbal therapy provides rational means for the treatment of many diseases such as respiratory problems, gastro-intestinal disorders, cardiovascular illness, metabolic and degenerative diseases/disorders associated with the aging. Research carried out on herbals in recent past has helped the society in the cure of certain cancers and search is continuing for finding remedies for AIDS and other diseases which so far are considered to be stubborn and incurable from any synthetic molecule [4].

Herbal bioenhancers are the substances which when mixed with the drug enhance the effectiveness of drug without taking away its properties. These agents at low doses promote and augment the bioavailability or biological activity of drugs. Their development is based on the ancient knowledge of Ayurveda. They are effective when used in combination with number of drug classes such as antibiotics, antidiabetic, antiasthmatic, antihyperlipedaemics, antifungal and anticancerous drugs. They improve bioavailability of wide range of nutrients such as vitamins, minerals and amino acids [5].

Modern researchers are increasingly showing interest towards the improvement of bioavailability of a large number of drugs by addition of various herbs with bioenhancing properties. Active components of natural compounds with bioenhancing properties such as piperine, quercetin, genistein, naringin, niazeridine, lysergols, capmul, sinomenine, glycyrrhizin, and nitrile glycoside are being isolated for their possible use along with modern medicines [6].

Treatment cost is a major concern for modern medicine in developing countries like India and systematic innovative means to reduce these costs are needed. Recent developments of another Ayurveda-based technology, this time, enhancing bioavailability of drugs, have produced a revolutionary shift in the way medicines are administered. The global focus is now on methods aimed at reducing drug dosage cost, reducing side effects and it will be beneficial in patients who need prolonged treatment. As a result, treatments are now becoming more affordable for wide sections of society, including the financially challenged and risk of tolerance/addiction will be reduced. One way to achieve reduction in drug dosage, and therefore drug toxicity and cost, is to increase drug bioavailability [7].

Materials and Methods

Experimental Animals

Wistar rats (25 to 30 g) of either sex were used for this study. They were maintained under standard conditions (temperature 22±2ºC, relative humidity 60±5% and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water ad libitum. All the animals received humane care according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the “National Academy of Sciences” and published by the “National Institute of Health”. All the procedures were performed in accordance with Institutional Animal ethics committee constituted as per the direction of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), under ministry of animal welfare division, Government of India, New Delhi, India.

Chemicals:
All the chemicals and reagents used were of analytical grade and were purchased from Yarrow Chem, Loba Chem, Himedia and Agappe diagnostics.

Methodology

Preparation of Ethanolic Extract of *Piper Nigrum* Seeds

Fresh seeds were collected locally and shade dried for few weeks. Dried seeds were ground in an electric grinder to obtain coarse seed powder. The powder was stored in an air tight container. The powdered material (150 g) was packed in Soxhlet extractor and extracted using ethanol as solvent. The temperature was maintained on an electric heating mantle with thermostat control. Appearance of colorless solvent in the siphon tube was taken as the termination of extraction. The extract was concentrated to syrupy consistency by using rotary flash evaporator. The concentrated extract was then air dried at room temperature and stored in air tight container in 2–8°C until used [8].

Routes of Drug Administration

The vehicle, standard drug and test drugs were administered orally with the help of an oral feeding needle.

Ehrlich Ascites Carcinoma (EAC) Induced Tumour Model

Tumour Cell Line and Inoculation

Ehrlich ascites carcinoma (EAC) cell line inoculated mice was supplied by division of radiology and toxicology, Manipal life science centre, Manipal University, India for the investigation. The EAC cells were maintained by serial transplantation in mice. Full grown tumour cell line was aspirated from the mouse peritoneum, washed thrice with 0.9% saline and suspended in saline. About 1×10^6 cells were injected intraperitoneally into a healthy mouse.

Induction of Experimental Tumour

Ehrlich ascites carcinoma cells were aspirated from the tumour bearing mice aseptically. The cells were diluted with phosphate buffer saline and 1×10^6 EAC cells in 0.3ml phosphate buffer were injected intraperitoneally to obtain ascetic tumour in mice. The EAC inoculated mice were divided into 4 groups consisting of 12 animals each and one more group without EAC inoculation served as normal control. A day of incubation was allowed for the multiplication of cells. All the treatments were given orally 24hrs after tumour induction and continued till the termination of the study. The protocols of the treatment are mentioned below:

Group I: Normal control, Saline (0.9% NaCl)
Group II: Tumour control, (EAC cells)
Group III: EAC cells + Mercaptopurine (5mg/kg)
Group IV: EAC cells + *Piper nigrum* (200mg/kg)
Group V: EAC cells + Mercaptopurine (5 mg/kg) + *Piper nigrum* (200mg/kg)

On 11th day, half of the animals (n = 6) in each group were anaesthetized and blood samples were collected by sino-orbital puncture in sterilized tubes for the estimation of various haematological parameters. The same animals were sacrificed by cervical dislocation for the estimation of liver biochemical parameters. Remaining animals were kept to observe the survival life span of the hosts.

The anti-tumour activity was measured with respect to the following parameters:

Tumour volume: Ascitic fluid was collected in a measuring cylinder by making a small incision on the skin near the intraperitoneal cavity. The packed cell volume was determined by centrifuging at 1000 rpm for 5 min.

Tumour cell count: The ascitic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the number of cells in the 64 small squares was counted.

Viable/Non-viable tumour cell count: The cells were stained with Tryphan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable and those that took the stain were nonviable. The viable and nonviable cells were counted.

Percentage increase life span (% ILS):

The effect on tumour growth was monitored by recording the mortality daily and percentage increase in life span (% ILS) was calculated.

Body weight:

Body weights of the experimental mice were recorded at the beginning (day 0) and sequentially on every alternative day during the treatment period.

Estimation of Hematological Parameters

(A) White blood cell count (WBC)

This involves counting of WBC by diluting a sample of blood with Turks fluid which destroys the red cells and stains the nuclei of the leucocytes. The cells are counted in Neubauer chamber. The composition of Turk's fluid is 3ml Glacial acetic acid, 1ml Gentian violet (1% solution in water) and 100ml of distilled water. The glacial acetic acid haemolyses RBCs without affecting the cell membrane of WBCs. Function of gentian violet is to stain the nuclei of WBC, also acts as a preservative [9].

Procedure

The blood was taken up to 0.5 mark in the WBC pipette and diluted with Turk's fluid up to 11 mark, mixed thoroughly. 1-2 drops were discarded and charged the diluted blood into counting chamber with cover slip. The cells were counted after five minutes under low power objective in the compound microscope. Total WBC in undiluted blood was calculated and expressed as cell x 10^3 /mm^3 blood.

(B) Red blood cell count (RBC)

The blood is diluted 200 times with RBC diluting fluid (Haeym's fluid) in a RBC pipette and the cells are counted in the counting chamber. Ideal fluid for diluting blood should be isotonic, and neither cause haemolysis nor crenation of red cells. The composition of Haeym's fluid is Sodium chloride 5g (provides isotonicity), Sodium Sulphate 2.5g (as anticoagulant), Mercuric chloride 25g (as preservative) and Distilled water 100 ml [9].

Procedure

The blood was taken up to 0.5 mark in the RBC pipette and diluted with Haeym's fluid up to 101 mark, mixed thoroughly. 1-2 drops were discarded and charged the diluted blood into counting chamber (hemocytometer) with cover slip. The cells were counted after 5 minute under high power objective in the compound microscope. Total number of RBC in undiluted blood was calculated and expressed as cell x 10^6/mm^3 blood.

(C) Haemoglobin (Hb) content

Haemoglobinometry refers to measurement of the concentration of Hb in blood. Estimation of haemoglobin is done by Sahli–Adam's method. The haemoglobin present in a measured amount of blood is converted by dilute hydrochloric acid into acid haematin, which in dilution is golden brown in colour. The intensity of colour depends on the concentration of acid haematin, which in turn depends on concentration of haemoglobin. The colour of the solution, after dilution with water is matched against golden brown tinted glass rods by direct vision. The readings were obtained as g/dl [9].

Procedure

Graduated tube was filled with 0.1N HCl up to 10 mark. Blood was pipetted up to 20 mark and transferred into the graduated tube of haemoglobinometer, containing 0.1N HCl. The solution in the graduated tube was diluted by adding distilled water drop wise until the colour matched with the standard colour strips.

(D) Differential leucocyte count (DLC)

DLC is the determination of the different types of white blood leucocytes present in blood. White blood cells are classified into various groups depending on their size, feature of the nucleus and features of cytoplasm. They include Monocytes, lymphocytes, Neutrophils, basophils and eosinophils. Leishmann stain is used to determine different types of white blood leucocytes of blood. Leishmann stain contains eosinate of methylene blue dissolved in acetone free methyl alcohol [9].

Procedure

Blood film was stained with 8 to 10 drops of Leishmann stain and allowed to stand for 2 minutes. Then few drops of distilled water were added to it and allowed to stand for few minutes. Stream of water directly on the smear was poured to flush off the excess staining and the smear was allowed to dry. A drop of cedar oil was placed on the smear and observed under oil immersion lens. Depending on the acidic and basic nature of the cell, they will take different dye colour and were differentiated on their morphological appearance also.

Estimation of Endogenous Antioxidant Parameters

Preparation of liver homogenate

The liver was quickly removed and perfused immediately with ice-cold saline (0.9% NaCl). A portion of the liver was homogenized in chilled Tris-HCl buffer (0.025M, pH 7.4) using a homogenizer. The homogenate obtained was centrifuged at 5,000 rpm for 10 min; supernatant was collected and used for analysis.

(A). Estimation of Reduced glutathione (GSH)

Procedure:

1g of scrapped tissue of the liver was homogenized with 10ml of 10% TCA in ice cold condition and centrifuged at 3000rpm for 10 minutes. The supernatant was separated. 2ml of 0.2M phosphate buffer of pH 8 was added to 0.5ml of supernatant. To the above solution 0.2ml of DTNB solution was added and absorbance was immediately measured at 412nm against blank. Increase in absorbance (optical density) is directly proportional to concentration of GSH; hence % Increase in GSH was calculated using the formula

% Increase in GSH = (Abs of control- Abs of test / Abs of control) x100

(B). Estimation of lipid peroxidation (MDA)

Procedure

Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS), using malondialdehyde (MDA) as standard. One ml of the liver homogenate (supernatant) was added to 2.0ml of the TBA-TCA HCl reagent. The contents were boiled for 15 minutes, cooled and centrifuged at 10000rpm to remove the precipitate. The Absorbance of supernant was determined against blank. Colour intensity of pink colour chromogen was measured at 535nm. Increase in the absorbance indicates greater the MDA conversely decrease in absorbance indicates lesser MDA indirectly lesser the lipid peroxidation. Hence the Absorbance was measured and % inhibition of lipid peroxidation was calculated using the formula % Decrease in LPO = (Abs of control- Abs of test / Abs of control) x100

(C). Estimation of Superoxide dismutase (SOD)

Procedure

To 0.4ml of the homogenate (supernatant), 1.2ml of sodium pyrophosphate buffer, 0.1 ml of Phenazine methasulphate (PMS), 0.3ml of nitro blue tetrazolium (NBT) and 0.8ml of distilled water was added to make up the volume up to 3ml. The reaction was started by addition of 0.3ml of NADH. It was incubated at 30°C for 60 sec. The reaction was stopped by addition of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4ml of n-butanol. The mixture was allowed to stand for 10 min, centrifuged and butanol layer was taken out. Colour intensity of the purple colour chromogen was measured against butanol at 560nm using spectrophotometer. Increase in absorbance (optical density) is directly proportional to concentration of SOD and vice versa and % increase in SOD was calculated.

% Increase in SOD = (Abs of control- Abs of test / Abs of control) x 100

(D). Estimation of catalase (CAT)

Procedure

The reaction mixture (1.5ml) contained 1.0ml of 0.01M pH 7.0 phosphate buffer, 0.1ml of tissue homogenate (supernatant) and 0.4ml of 2M H2O2. The reaction was stopped by the addition of 2.0ml of dichromate-acetic acid

reagent and was incubated at 100°C for 2 min. Colour intensity was measured colorimetrically at 620nm. Increase in absorbance is directly proportional to concentration of CAT. Hence % increase in CAT was calculated using the formula:

% Increase in CAT = (Abs of control- Abs of test / Abs of control) x 100

Statistical Analysis

All data were expressed as Mean ± SEM. The statistical significance between groups were compared using One way ANOVA, followed by Dunnett's multiple comparison test.

Results

Effect on Haematogical parameters

Haemoglobin content and RBC count were significantly decreased and total WBC count was significantly increased in the tumour group as compared to the normal group. In the differential count of WBC, the Neutrophils count increased, while the lymphocyte count decreased in the tumour control group as compared to the normal group. Treatment with both Mercaptopurine (5mg/kg) and *Piper nigrum* (200mg/kg) alone restored the altered parameters towards the normal values but combination therapy of Mercaptopurine along with *Piper nigrum* showed statistically very significant effects as summarized in Table 1.

Table 1: Effect of Mercaptopurine in combination with *Piper nigrum* on Haematogical parameters of Ehrlich ascites carcinoma bearing mice

Treatment	Hb (g/dl)	Total RBC (10^6 cells/mm^3)	Total WBC (10^3cells/mm^3)	Differential count		
				% Mono cytes	% Neutro Phils	% Lympho cytes
Normal control (Saline)	13.03 ± 0.24	7.29 ± 0.18	5.66 ± 0.15	2.04 ± 0.03	15.31 ± 0.16	85.49 ± 0.14
Tumour control (EAC cells)	8.03 ± 0.13	3.07 ± 0.26	3.07 ± 0.26	1.16 ± 0.04	75.04 ± 0.16	24.69 ± 0.09
EAC cells + *Piper nigrum* (200mg/kg)	10.01 ±0.14**	5.01 ± 0.28**	4.21 ± 0.06**	1.42 ± 0.03*	27.40 ± 0.19**	65.59 ± 0.12**
EAC cells + Mercaptopurine (5mg/kg)	12.33 ± 0.33***	6.39 ± 0.22***	5.07 ± 0.21***	1.58 ± 0.08***	21.58 ± 0.08***	80.62 ± 0.09***
EAC cells + Mercaptopurine (5 mg/kg) +*Piper nigrum* (200mg/kg)	12.90 ± 0.23***	7.24 ± 0.28***	5.54 ± 0.21***	1.64 ± 0.09***	18.64 ± 0.09***	83.60 ± 0.11***

Values are expressed as Mean ± SEM. n = 6 for each group. *p <0.05, **p<0.01, ***p<0.001.
One way ANOVA followed by Dunnett's test compared to Tumour control.

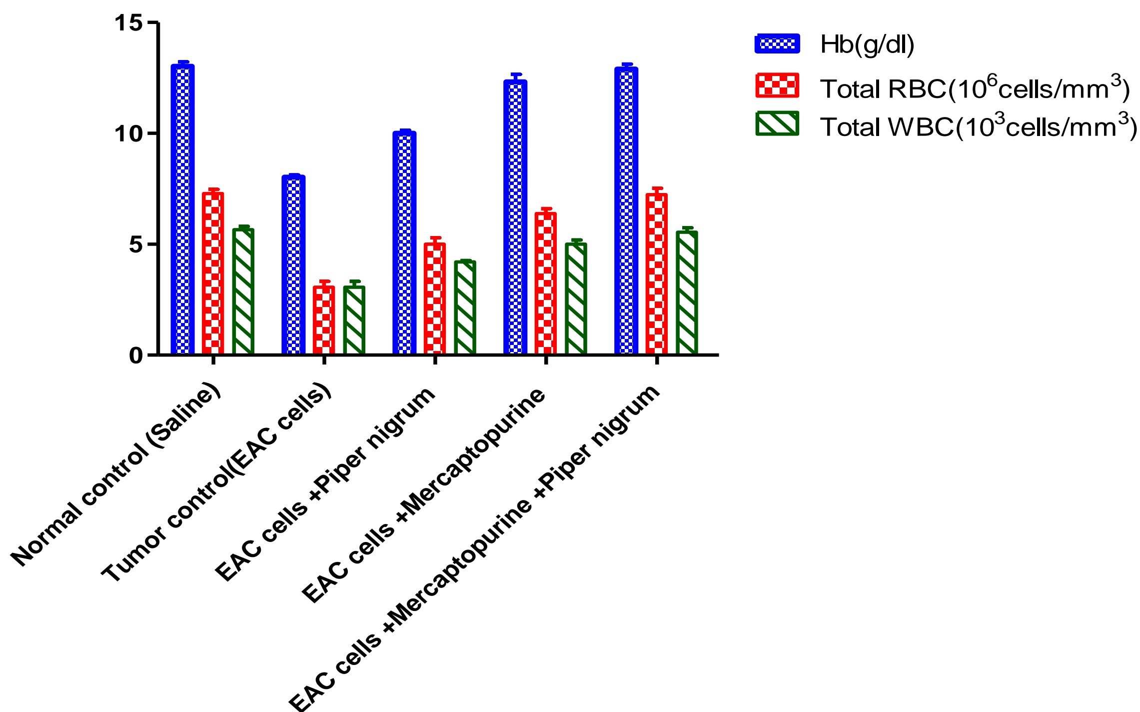

Figure 1: Effect of Mercaptopurine in combination with Piper nigrum on Haematogical parameters of Ehrlich ascites carcinoma bearing mice

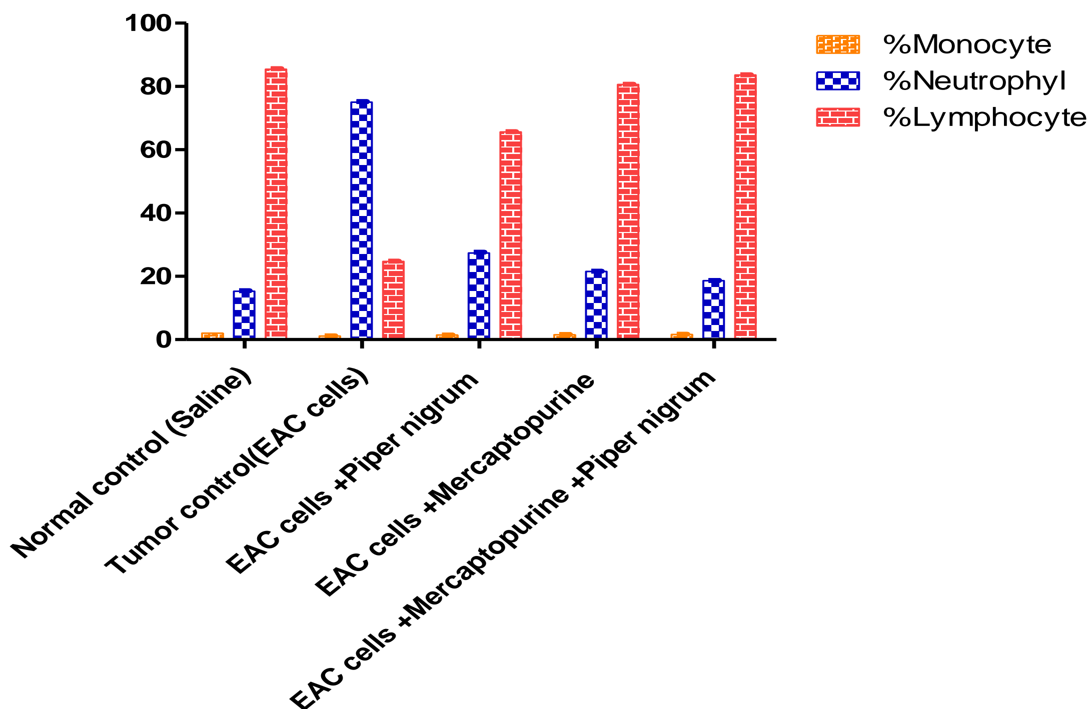

Figure 2: Effect of Mercaptopurine in combination with Piper nigrum on Haematogical parameters of Ehrlich ascites carcinoma bearing mice

Effect on enzymatic antioxidants parameters

Lipid peroxidation levels indicated by TBARS were considerably higher in untreated EAC bearing animals as compared to the normal animals. Inoculation with EAC cells results in drastic decrease in the activities of glutathione and enzymatic antioxidants (SOD and CAT). Treatment with Mercaptopurine (5mg/kg) alone resulted in a significant decrease in levels of TBARS and brought them near to normal level. The activities of glutathione and enzymatic antioxidants were also significantly reversed to near normal values. In the same way *Piper nigrum* at the dose of 200mg/kg alone was found to be less effective in normalising the altered parameters. Mercaptopurine when co administered along with *Piper nigrum* showed significantly better effects as summarized in **Table 2**

Table 2: Effect of Mercaptopurine in combination with *Piper nigrum* on LPO, GSH, SOD, and CAT levels in Ehrlich ascites carcinoma bearing mice

Treatment	LPO	GSH	SOD	CAT
Normal control (Saline)	0.065 ± 0.012	0.396 ± 0.016	1.552 ± 0.037	0.545 ± 0.05
Tumour control (EAC cells)	0.431 ± 0.036	0.199 ± 0.09	0.093 ± 0.01	0.233 ± 0.02
EAC cells + *Piper nigrum* (200mg/kg)	0.298 ± 0.019**	0.285 ± 0.034*	0.321 ± 0.026**	0.342 ± 0.014**
EAC cells + Mercaptopurine (5mg/kg)	0.174 ± 0.012***	0.317 ± 0.018**	0.517 ± 0.018**	0.374 ± 0.01***
EAC cells + Mercaptopurine (5 mg/kg) + *Piper nigrum* (200mg/kg)	0.154 ± 0.025***	0.354 ± 0.015***	0.691 ± 0.041***	0.412 ± 0.01***

Values are expressed as Mean ± SEM. n = 6 for each group*p<0.05, **p<0.01, ***p<0.001. One way ANOVA followed by Dunnett's test compared to Tumour control.

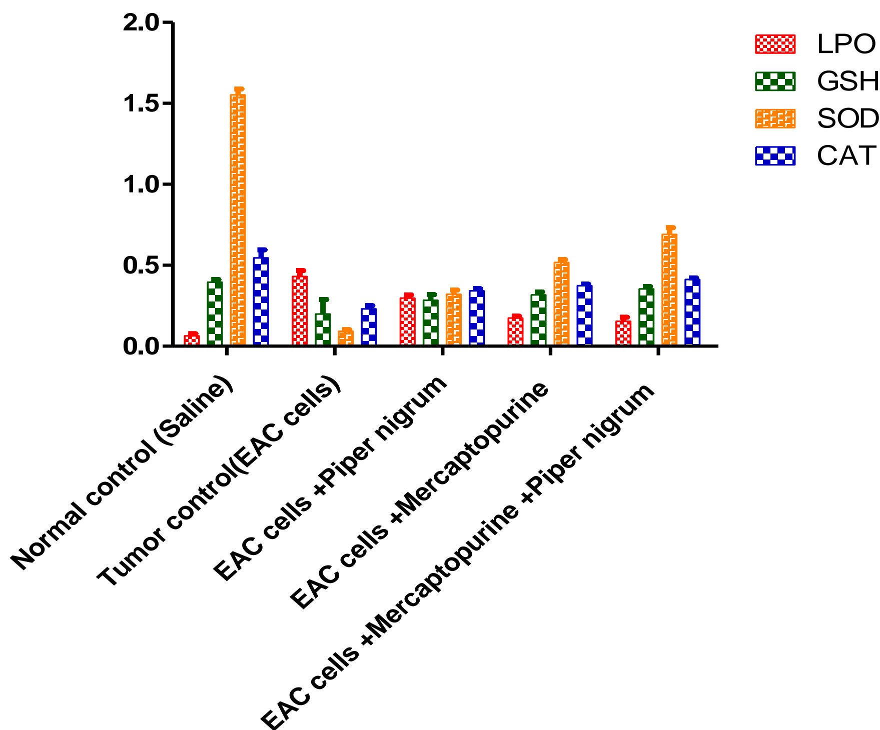

Figure 3: Effect of Mercaptopurine in combination with Piper nigrum on LPO, GSH, SOD and CAT levels in Ehrlich ascites carcinoma bearing mice

Effect on Mean survival time, %increase in life span and Average Increase in Body Weight

The effect of Mercaptopurine in combination with *Piper nigrum* on the Mean survival time (MST) was monitored. Tumour bearing animals treated with Mercaptopurine (5mg/kg) and *Piper nigrum* (200mg/kg) individually, shown a increase in survival period but the effect was more significant in animals treated with combination therapy of Mercaptopurine (2.5mg/kg) along with *Piper nigrum.* Finally, the change in body weights of the animals suggests the tumour growth inhibiting property of the drugs. All these results clearly indicate that the Mercaptopurine (5mg/kg) + *Piper nigrum* (200mg/kg) has a remarkable capacity to inhibit the growth of tumour induced by EAC cell line. The results are summarized in **Table: 3**

Table 3: Effect of Mercaptopurine in combination with *Piper nigrum* on Mean survival time, % increase in life span and % increase in body weight of EAC tumour bearing mice

Treatment	Mean Survival Time (days)	% Increase in life span	% Increase in body weight
Tumour control (EAC cells)	23.02± 2.09	----	----
EAC cells + *Piper nigrum* (200mg/kg)	24.04 ± 2.16**	4.40 %	27.20 ± 0.31*
EAC cells + Mercaptopurine (5mg/kg)	31.30 ± 3.14***	43.53 %	15.89 ± 0.30**
EAC cells + Mercaptopurine (5mg/kg) + *Piper nigrum* (200mg/kg)	36.18 ± 2.04***	71.38 %	10.89 ± 0.30**

Values are expressed as Mean ± SEM. n = 6 for each group. *p<0.05, **p<0.01, ***p<0.001. One way ANOVA followed by Dunnett's test compared to Tumour control.

Effect on Tumour volume, packed cell volume, viable and non-viable cells of EAC bearing mice

Treatment with Mercaptopurine (5mg/kg) and *Piper nigrum* (200mg/kg) alone decreased the tumour volume, packed cell volume and viable cell count whereas non-viable cell count was higher in treated animals compared to tumour control animals. The combination therapy shown much higher significant effect compared to when administered individually. The results are summarized in Table: 4

Table 4: Effect of Mercaptopurine in combination with *Piper nigrum* on Tumour volume, packed cell volume, viable and non-viable cells of EAC bearing Mice

Treatment	Tumour Volume (ml)	Packed cell Volume (ml)	Viable Cells ($X10^6$cells/ml)	Nonviable cells ($X10^6$cells/ml)
Tumour control (EAC cells)	4.17 ± 0.06	2.55 ± 0.15	8.46 ± 0.15	0.82 ± 0.01
EAC cells + *Piper nigrum* (200mg/kg)	3.34 ± 0.08*	1.83 ± 0.03**	7.90 ± 0.14*	1.22 ± 0.04*
EAC cells + Mercaptopurine (5mg/kg)	2.01 ± 0.26**	1.04 ± 0.05***	4.57 ± 0.16**	2.37 ± 0.10***
EAC cells + Mercaptopurine (5mg/kg) + *Piper nigrum* (200mg/kg)	1.35 ± 0.12**	0.55 ± 0.09***	1.33 ± 0.11**	4.13 ± 0.05***

Values are expressed as Mean ± SEM. n = 6 for each group.*p<0.05, **p<0.01, ***p<0.001.One way ANOVA followed by Dunnett's test compared to the Tumour control.

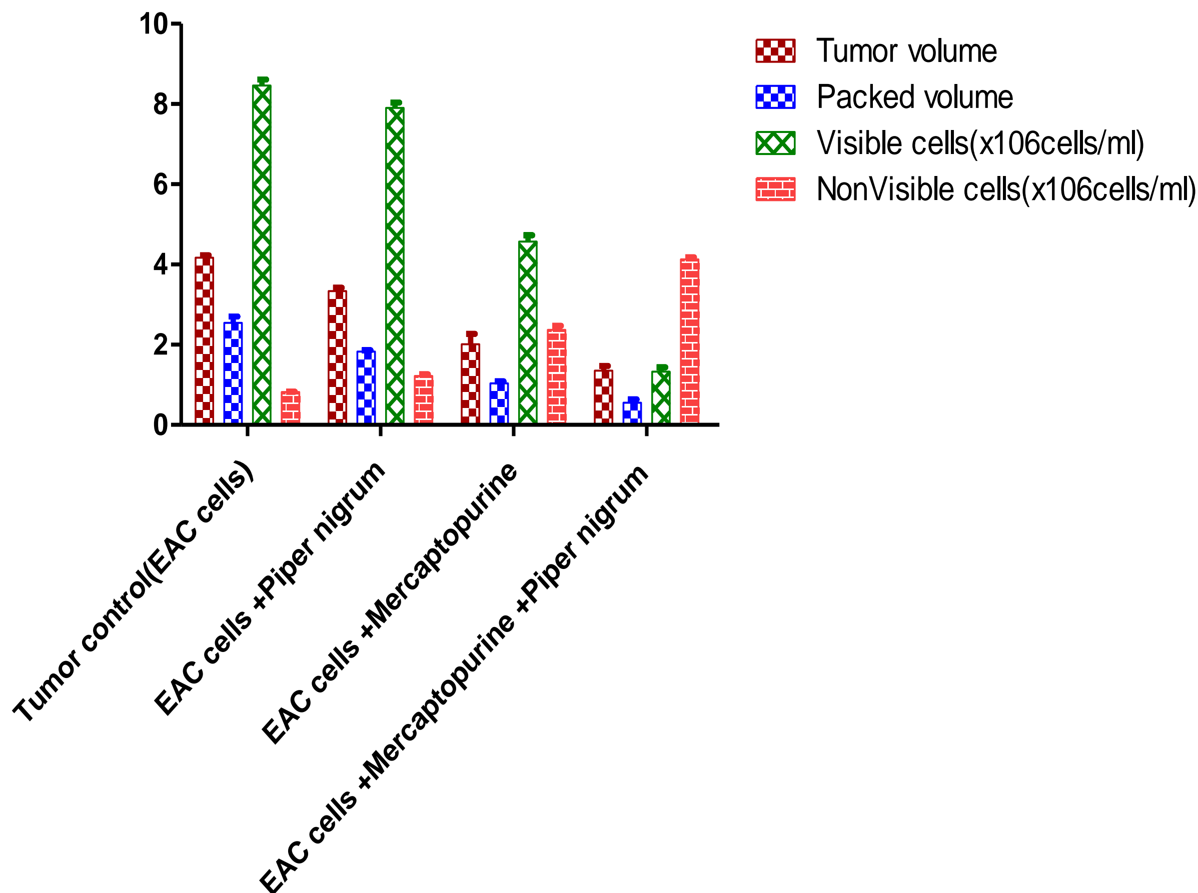

Figure 4: Effect of Mercaptopurine in combination with Piper nigrum on Tumour volume, packed cell volume, viable and non-viable cells of EAC bearing Mice

Discussion

Cancer is one of the most life threatening diseases and serious health problem in both developing and developed countries. Many synthetic and chemotherapeutic agents used in cancer therapy involve the risk of host toxicity. Mercaptopurine is one of the antimetabolite, antineoplastic agent with immunosuppressant properties. Mercaptopurine has been widely used in the treatment of certain types of cancer, human leukaemia and inflammatory bowel disease. One or more of the biochemical effects of mercaptopurine and its metabolites are directly or predominantly responsible for cell death. The bioavailability of oral mercaptopurine at standard dose is very low, largely as a result of extensive first-pass metabolism by xanthine oxidase. Treatment with Mercaptopurine is associated with many of the severe adverse side effects which are dose related [10].

Natural compounds have been expected to play an important role either as chemo preventive or chemotherapeutic agents to fight against cancer. Modern researchers are increasingly showing interest toward the improvement of bioavailability of a large number of drugs by addition of various herbs with bioenhancing properties. Bioavailability and absorption enhancement through co-administration of drugs with naturally occurring compounds from plants are considered to be very simple and relatively safe. In oral drug delivery system, the co-administration of therapeutic agents with natural compounds possessing absorption improving activities, has also garnered great interest [11].

The present work aimed to study the antitumour effect of mercaptopurine in combination with *Piper nigrum* in experimentally induced carcinogenesis. The reliable criteria for judging the value of any anticancer drug are prolongation of lifespan and disappearance of leukaemic cells from the blood. Treatment with mercaptopurine in

combination with *Piper nigrum* demonstrated very significant reduction in WBC from blood of tumour bearing mice It can therefore be inferred that mercaptopurine in combination with *Piper nigrum* increased the life span of tumour bearing mice by preventing tumour progression [12].

Usually the major problems in cancer chemotherapy are Myelosuppression and anaemia. The anaemia encountered in tumour bearing mice is mainly due to reduction in RBC or haemoglobin percentage and this may occur either due to iron deficiency or due to haemolytic or myelopathic conditions. Present study indicates that combination therapy with mercaptopurine along with *Piper nigrum* restored back the haemoglobin content and RBC count more or less to normal levels. This indicates that the test compounds possess protective action on haemopoietic system. These observations assume great significance as anaemia is a common complication in cancer and the situation aggravates further during chemotherapy since a majority of antineoplastic agents exert suppressive effects on erythropoiesis and thereby limiting the use of drugs [13].

Free radicals and reactive oxygen species (ROS) are continuously produced in the human body. These oxygen species are the cause of cell damage and the progression of normal cells to cancer cells. Therefore, tissues must be protected from oxidative injury through intracellular as well as extracellular antioxidants. Elevated lipid peroxidation causes tissue injury and damage to cellular macromolecules by the generation of reactive oxygen species (ROS), increasing the risk for cancer. ROS formed during carcinogenesis tissues result in lipid peroxidation and subsequently increase in MDA and other TBARS levels. MDA, the end product of lipid peroxidation, a biomarker of oxidative stress, was reported to be higher in cancer tissues than in non-diseased organs. The high levels of MDA in cancerous conditions could result from the deterioration of antioxidant defences. This study showed that TBARS levels measured as MDA in the tumour bearing liver tissues were higher than the normal saline treated liver tissues. Treatment with Mercaptopurine along with *Piper nigrum* inhibited hepatic lipid peroxidation as revealed by the reduction of MDA levels toward normal levels. Polycyclic aromatic hydrocarbons induce the formation of ROS and subsequent degradation of cellular defences. Decrease in lipid peroxidation by Mercaptopurine and *Piper nigrum* in this study implies that its chemo preventive action is presumably mediated by preventing or reducing the DNA damage by oxidative free radicals. Glutathione, a potent inhibitor of the neoplastic process, plays an important role in the endogenous antioxidant system. It is found in particularly high concentration in the liver and is known to have a key function in the protective process. The free radical scavenging system, superoxide dismutase and catalase are present in all oxygen metabolising cells and their function is to provide a defense against the potentially damaging reactive of superoxide and hydrogen peroxide. Decrease in SOD activity in tumour bearing animals, which might be due to loss of Mn^{2+} containing SOD activity in tumour cells and loss of mitochondria, leading to a decrease in total SOD activity in the liver. The inhibition of SOD and CAT activities as a result of tumour growth was reported. Decrease in SOD, GSH and CAT activities described in tumour is regarded as markers of malignant transformation. Similar findings were observed in tumour induced mice. Treatment with Mercaptopurine in combination with *Piper nigrum* very significantly elevated the reduced hepatic SOD, CAT and GSH levels in tumour bearing mice [14].

The lowering of lipid peroxidation and increase in levels of SOD, CAT and GSH in treated groups indicates its potential as an inhibitor of tumour induced intracellular oxidative stress. Antitumour activity of these antioxidants is either through induction of apoptosis or by inhibition of neovascularisation. The involvement of free radicals in tumours is well documented. The Ehrlich Ascitic tumour implantation induces *per se* local inflammatory reaction, with increasing bioavailability and hence bioefficacy of novel classes of drugs can be increased by herbal bioenhancers. In today's era, there is a greater interest and larger healthcare need for the enhancing of bioavailability of a many drugs which are less bioavailable. Poorly bioavailable drugs remain sub-therapeutic because a larger portion of a dose never reaches the site of action and shows its biological effect. A larger doses are required which may lead to serious adverse effects. Improvements in bioavailability can results into lowering the dose and also the dose frequency of the drug [15].

India being a developing country, cost of treatment is a real concern for new allopathic medicine. Innovative methods to reduce these costs of medicine are present demand. Bioenhancers highlights the benefits of integrating of an ancient system with present modern system of medicine in practice. The complementary action of bioenhancer

can reduce the dose of rifampin to half, patients need to pay very less of the original treatment This may leads to tremendous economic benefit for the tuberculosis suffering world population. Also if bioenhancer action is applied to other drugs, benefit levels become astonishing. Internationally, many billions of dollars are spent annually due to the poor bioavailability of many drugs [16].

The present study was undertaken to evaluate the antidiabetic activity of a standard synthetic antidiabetic drug glibenclamide in combination with an herbal bioenhancer drug ginger in diabetic rats. In the present study, diabetes was induced using streptozotocin (STZ). Streptozotocin is a broad spectrum antibiotic, induces diabetes in a wide variety of animal species by damaging the insulin-secreting cells of the pancreas [12].

Glibenclamide is a second generation sulphonylurea derivative, oral hypoglycemic agent and found to be effective in diabetic rats that retain functioning of islet β-cells. Hence the principle mechanism of action is to stimulate the production and secretion of insulin by the β-cells of pancreas. This drug may lower down the output of glucose from the liver by insulin independent mechanism. Induction of diabetes is associated with a characteristic loss of body weight, which is due to increased muscle wasting and loss of tissue proteins [18].

Insulin deficiency leads to various metabolic aberrations in the rats; the rise in blood glucose level is accompanied by increase in SGPT and SGOT level. Oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. Free radicals are formed in diabetes by glucose oxidation, protein glycation and the subsequent degradation of glycated proteins. High levels of free radicals and the simultaneously declined antioxidant enzyme levels lead to cell damage, inactivation of enzymes and lipid peroxidation. Superoxide dismutase and catalase play an important role in the detoxification of super oxide anion and H_2O_2 respectively. In present study, Catalase and SOD which are most important antioxidant enzymes were found to be decreased in diabetic control group. Treatment with glibenclamide and glibenclamide + ginger extract combination restores the level of both enzymes [19].

Conclusion

Adverse effects of Mercaptopurine like Hyperuricaemia, bone marrow toxicity, hypoplasia, anorexia, diarrhoea, leukopenia, thrombocytopenia, Intestinal ulceration, crystalluria with haematuria, immunosuppression, Interstitial pneumonitis, cutaneous hyperpigmentation, alopecia, Myelosuppression, hepatotoxicity, cholestatic jaundice etc are usually dose related.

Piper nigrum if co-administered along with mercaptopurine augments the bioavailability. Thus if mercaptopurine given along with bioenhancer like *Piper nigrum* dosage of mercaptopurine can be reduced. Also treatment becomes cost effective, minimizing drug toxicity and adverse reactions.

The results indicate mercaptopurine in combination with *Piper nigrum* showed antitumour activity which is more significant than when they are administered alone. The study provides a research tool to improve bioavailability of a large number of drugs by addition of various herbs with bioenhancing properties.

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