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Anticancer activity of curcumin alone and in combination with piperine in Dalton lymphoma ascites bearing mice

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Curcumin has been reported for its anticancer activity, but clinically it suffers from low bioavailability. In this context, we explored the potential of a natural bioavailability enhancing agent piperine in the present study. Piperine too has anticancer activity, and thereby combination of these two natural ingredients were tested for better therapeutic use. Curcumin (50 mg/kg, 100 mg/kg), piperine (10 mg/kg) alone and in combination was evaluated in Dalton lymphoma ascites (DLA) bearing mice by assessing various biochemical and histopathological parameters. Treatment with the curcumin at two different concentrations and piperine alone has shown some therapeutic benefit in reducing the tumors and increased the lifespan of the tested animals (%ILS). The treatment groups have shown significant therapeutic benefits in restoration of hematological and biochemical parameters, particularly in combination treatment groups. Precisely, curcumin and piperine in combination have shown more significant influence in the restoration of various hematological and biochemical parameters. Histopathological observations also revealed that combination of curcumin and piperine improves repairing of the tissue damage due to inoculation of lymphoma significantly.

Keywords: Black pepper, Cancer therapeutics, *Curcuma longa*, Long pepper, Lymphoma, *Piper longum*, *Piper nigrum*, Tumor, Turmeric

Cancer is one of the major non-communicable diseases affecting 18.1 million patients worldwide[1]. It is reported to be the second most lethal disease in the United States despite 29% drop in the mortality rate from 1991 to 2017[2]. The Federal Government has spent $147.3 billion on cancer care in 2017, and it is estimated to increase in view of the expected rise in new cases to 23.6 million by 2030[3]. In the Age-Standardized Rate (ASR) of cancer incidence and mortality globally (24 world areas) for all cancers combined, India ranks 18th in cancer incidence with ASR 279.8 and the least (24th) in mortality with ASR 123[1]. The present study focuses on lymphoma cancer, a heterogeneous group of malignant disease with a wide spectrum of illnesses, comprising 70 different subtypes, and observed most commonly in children, next only to Leukemia[4,5]. Currently, the Non-Hodgkins lymphoma (NHL), with the new cases of 509590 in 2018, ranks 13th in cancer incidence[1]. The estimated rate of incidence of NHL in 2012 for India was 22 and mortality 1.5 per million population 2.2, and mortality 0.15 per 0.1 million population[6]. Asia is reported to lead both in cancer incidence (57.3%) as well as in cancer mortality (48.4%)[1].

Though we have different strategies for the treatment of cancer viz., radiotherapy, endocrine therapy, immunotherapy and chemotherapy, most of them are associated complications such as bone marrow depression, organ toxicity, alopecia and drug-induced cancers[7]. Though the chemotherapy has shown promising results irrespective of the stages of cancer, it has limitations like non-selectivity and multidrug resistance[8]. The above challenges have encouraged researchers to develop innovative methods to resolve the issues and also screen natural sources for possible potential alternative medicines to treat cancer effectively with 'nil or low' side effects[9-11].

Curcumin is one of the natural polyphenolic compounds obtained from *Curcuma longa* L. and it has been well known as a dye, flavouring and a medicinal agent for thousands of years. Traditionally, it is used for its antioxidant, anti-inflammatory, antiobesity, antiseptic, antitumor, antiplatelet, hepato- and immuno-protetive, and chemopreventive activities[12-16]. It has been shown that curcumin and its

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derivatives increased the cell death in a wide variety of cancers like brain tumors[17], breast cancer[18], ovarian cancer[19], testicular cancer[20] and lymphoma[21]. However, curcumin suffers from low bioavailability, and hence require bioavailability enhancing agents for optimal result. Piperine, a known anticancer natural alkaloid from *Piper nigrum* (Black Pepper) and *P. longum* (Long Pepper), has been reported to enhance curcumin potential when used in combination[22,24]. In the present study, we observed the anticancer activity of curcumin alone and in combination with piperine on DAL (Dalton Ascites Lymphoma) lymphoma-bearing mice.

Materials and Methods

Collection and authentication of plant material

Piper nigrum L. fruits were collected from the fields of Bengaluru, authenticated by Dr. P. Satyanarayana Raju, plant taxonomist, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur.

Extraction of piperine

The dried seed was powdered with the miller, and the powder was extracted with ethanol (95%) by using a Soxhlet apparatus for 3 h. The solution was concentrated at 65°C, andadded with 10% alcoholic potassium hydroxide with constant stirring, and was filtered. The filtrate was kept aside for overnight and the crystals of piperine were separated from the flask[24]. The sample was characterized in FT-IR as described in Saha *et al*.[25].

Chemicals

Curcumin was obtained from Laila Nutraceuticals, Vijayawada; 5-fluoro uracil and Trypan Blue were obtained from Sigma Chemicals Ltd., India. All the other chemicals were obtained from Himedia and SD Fine Chemical, Mumbai, India.

Animals and treatment

Swiss albino mice 20-25 g were procured from Biogen, Bangalore, and they were maintained in the animal house of Acharya Nagarjuna University College of Pharmaceutical Sciences. All the animals were provided with standard pellet rat chow and water *ad libitum* and they were maintained at 12 h dark-light cycle under room temperatures (22°C±2). The experimental protocol was approved by the Institutional Animal Ethicks Committee (IAEC) of Acharya Nagarjuna University College of Pharmacy. DAL cell lines were obtained from the Amala Cancer Research Institute, Thrissur. They were injected into female mice with 1×10^6 cells in 0.1 mL for mice and the cell lines were maintained by serial intraperitoneal (i.p.) transplantations into female mice.

Transplantation of tumor cell lines

For transplantation of tumor cells, they were aspirated into a syringe aseptically and then washed with phosphate buffer solution (PBS) twice. The washed cells were diluted as 1×10^6 cells in 0.1 mL of PBS and they were transplanted into another animal[26].

Selection of test doses

Test doses of curcumin[27] with two different concentrations and a single dose of piperine[28] were selected from the previous literature as 50, 100 and 10 mg/kg, respectively.

Experimental design for DLA cell line

In this study, we used 96 female Swiss albino mice and they were maintained under laboratory condition for 1 week for acclimatization. All the animals were divided into 8 groups, each comprising 12 animals. On the first day of study protocol except for Gr. I, all the groups of animals were aseptically transplanted with DLA cells from the DLA bearing mice through i.p. The standard treatment 5-fluorouracil (5-FU) was given through i.p. route. Curcumin and piperine were administered through per os (p.o) route. The protocol was as follows: Group I, saline @5 mL/kg, p.o.;Group II, only vehicle @5 mL/kg, p.o.; Group III, 5-fluorouracil (5-FU) @20 mg/kg i.p. as standard drug; Groups IV & V, curcumin @50 and 100 mg/kg, p.o., respectively; Group VI, piperine @10 mg/kg, p.o.; and Groups VII & VIII piperine @ 10 mg/kg, p.o. with curcumin @ 50 and 10 mg/kg, p.o., respectively.

Except for groups I-III, the rest were treated with test doses one week prior to DLA transplantation and continued for next 14 days. The test doses were suspended and the suspension was given orally. For Gr. I-III, the treatment had started on the first day of tumor transplantation and continued for 14 days of the protocol. On the last day, 6 animals from each group were anesthetized with diethyl ether to collect the blood samples for estimation of hematological and serum biochemical parameters. Peritoneal tumor fluid was collected with 18 gauze syringes for the determination of tumor volume, packed cell volume and viable cell count. They were sacrificed to collect the liver samples for *in vivo* antioxidant studies. The remaining animals from each group were observed for

mean survival time (MST) and percentage increase in life span (%ILS) for 50 days[29].

Tumor cell count

The Viable cell count was observed with the trypan blue assay. In this assay, we have used a 0.4% trypan blue working solution and this solution was added to the cell suspension, kept it aside for 3 min for staining. The cells, which attain blue stain were non-viable and those cells without stain were viable cells. Then viable cell count was performed in the hemocytometer[30].

Tumor volume

The entire ascetic fluid was collected with the help of 18 gauge syringe into a centrifuge tube and measured the volume of ascitic fluid from each animal[29].

Animal weights

All the animals were weighed before and after the withdrawal of ascetic fluid on the 15th day to measure the tumor weight[29].

Hematological parameters

The collected blood was used for estimation of different hematological parameters like red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb) using laboratory procedures[29].

Mean survival time and percentage increase in life span

The remaining 6 animals from each group were observed for 50 days for their mean survival time in order to calculate the percentage increase in life span[29].

Serum biochemical parameters

The serum collected from the animals earlier was used for estimation of alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) with the help of Span Diagnostic kits using Semi autoanalyzer.

***In vivo* antioxidant studies**

Homogenization of liver tissue

Liver samples from the animals were washed with PBS and then minced into small pieces. Then 1.0 g of tissue sample was homogenized in ice-cold 10% trichloroacetic acid solution.

Lipid peroxidation

Lipid peroxidation was performed for the tissue homogenate by Ohkawa H method. To 1.0 mL of the tissue homogenate we have added 2 mL of TCA-TBA-HCL reagent (15% w/v trichloroacetic acid; 0.375% w/v thiobarbituric acid; 0.25 N hydrochloric acid). The solution was mixed thoroughly and then kept for heating in a boiling water bath for 15 min. After cooling the homogenate was centrifuged to get supernatant and the absorbance of the supernatant was measured at 535 nm[31].

Glutathione estimation:

The reduced glutathione (GSH) estimation was performed for the tissue homogenate by minor modification in the Ellman method. The homogenate was centrifuged in cold centrifuge at 3000 rpm for 15 min and the 0.5 mL of supernatant was collected. The supernatant was added to 2 mL of 0.3 M disodium hydrogen phosphate solution, to that 0.2 mL of DTNB (dithiobisnitrobenzoate, 0.4 mg/mL of sodium citrate) was added. The absorbance was measured at 412 nm[32].

Histopathology

Liver samples from each group were used for histopathological studies. They were kept in 10% formalin solution and were washed and dried in different solvents. The samples were embedded in paraffin blocks and they were sectioned into 5µ sizes which were used for staining with Eosin and Haematoxylin[33].

Statistical analysis

Data was projected as Mean±SD. To analyze the data we did One-way ANOVA method followed by Tukey's comparison and the P <0.05 was considered as a significant result.

Results

FT-IR analysis of piperine

The extracted piperine was analyzed by the FT-IR spectral analysis. The results are shown in Fig. 1 which indicates peaks at 2936 cm^{-1} 2849 cm^{-1} 1631 cm^{-1} 1609 cm^{-1} 1578, 1488 cm^{-1} 1247, 1106 cm^{-1} corresponding to C-H (Ar), C-H, C=O, C=C (Diene), C=C (Phenyl Ring), and C-O-C, respectively.

Effect on animal weights, tumor volume, and viable tumor cell count

A significant (P <0.001) increase in animal body wt. was observed in tumor control mice when compared with normal control animals. The 5-FU treatment group has shown significant (P <0.001) results in decreasing the body wt. In the treatment groups with curcumin 50 and 100 mg/kg was found to be a significant reduction in body wt. (P <0.05, P <0.01), respectively. The piperine alone treated group also has shown significant (P<0.05) reduction in body wt. The combination groups of curcumin 50 and

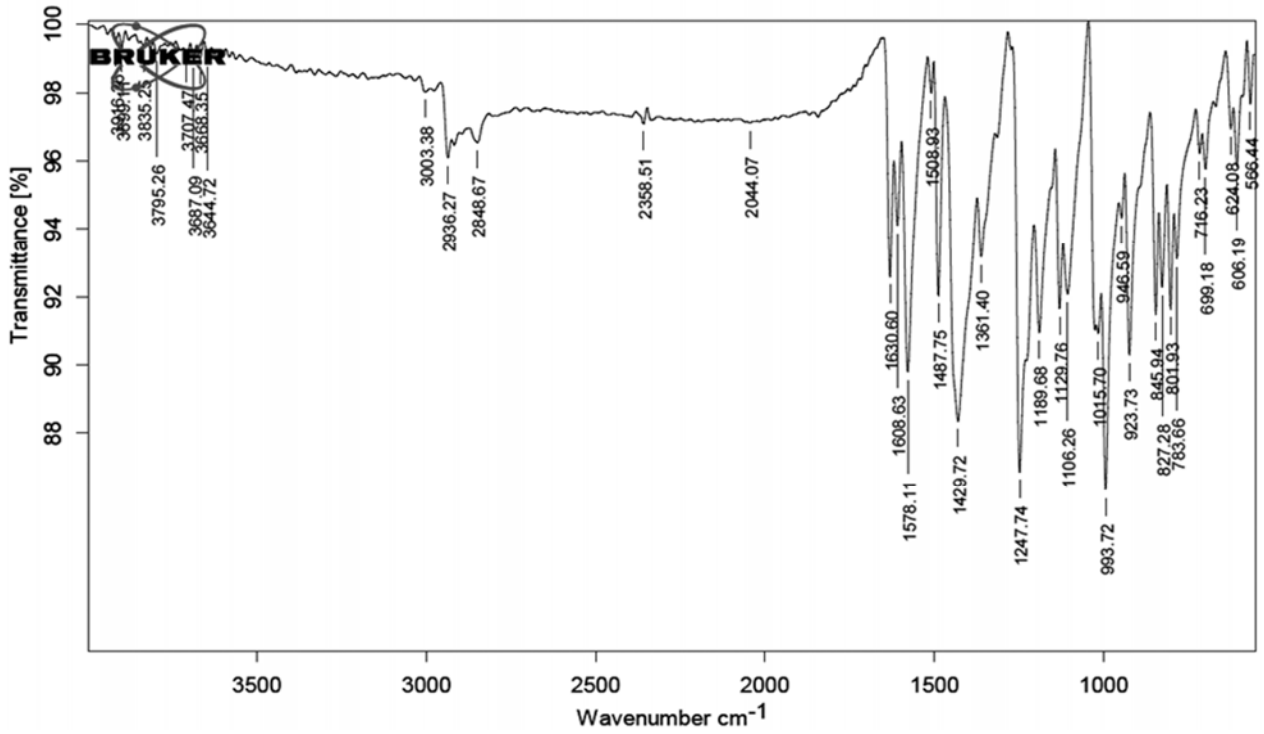

Fig. 1 — FT-IR analysis of piperine. [Peaks at 2936 cm^{-1} 2849 cm^{-1} 1631 cm^{-1} 1609 cm^{-1} 1578, 1488 cm^{-1} 1247, 1106 cm^{-1} corresponding to C-H (Ar), C-H, C=O, C=C (Diene), C=C (Phenyl Ring), and C-O-C, respectively]

100 mg/kg with piperine 10 mg/kg (VII & VIII) were found to be more significant (P <0.001) than the individual treatment groups.

The 5-FU treatment group showed decreased tumor volume significantly (P <0.001) when compared with the tumor control group. Gr. IV (curcumin, 50 mg/kg) didn't show any significant decrease in tumor volume, while Gr. V (100 mg/kg) showed a significant decrease in tumor volume (P <0.05) compared to the tumor control. Piperine 10 mg/kg alone (Gr. VI) also showed significant (P <0.05) decrease in tumor volume. The combination groups (Gr. VII & VIII) too had more significant (P <0.001) decrease in tumor volume than individual groups.

The 5-FU treated group was shown significant (P <0.001) decrease in viable cell count when compared with the tumor control group. The curcumin 50 mg/kg group (Gr. IV) didn't show any significant difference in viable cell count with the tumor control group, whereas Gr. V (curcumin 100 mg/kg) exhibited significant (P <0.01) decrease in tumor cell count compared to the tumor control. Gr. VI (piperine 10 mg/kg) also showed significant (P <0.05) decrease in tumor cell count. Groups VII & VIII (combinations of curcumin 50 and 100 mg/kg with piperine 10 mg/kg) have also shown more significant (P<0.001) decrease in viable tumor cell count compared to the tumor control.

Table 1 — Effect on animal weights, tumor volume and viable tumor cell count

Groups	Animal weights (g)	Tumor volume (mL)	Tumor cell count
I	2.85±0.63	-	-
II	10.67±0.39***	8.28±0.63	3.602±0.50
III	4.30±0.64***	2.50±0.46***	1.358±0.36***
IV	8.99±0.31*	6.83±0.73ns	3.183±0.48ns
V	8.65±0.39**	6.52±0.92*	2.757±0.33**
VI	8.91±0.37*	6.76±0.99*	2.920±0.14*
VII	6.60±0.80***	5.90±0.68***	2.063±0.26***
VIII	5.10±0.64***	4.03±0.99***	1.735±0.19***

[Results are expressed as Mean ± SD; n=6 in each group; ***significantly different at P <0.001, ** significantly different at P <0.01, * significantly different at P <0.05, ns not significant]

The effect of curcumin, piperine alone and in combination on animal weights, tumor volume and viable cell count are summarized in Table 1.

Effect on percentage increase in life span (% ILS) and hematological parameters

The MST was found decreased to 20.5 days in the tumor control group while the treatment groups IV & V with curcumin 50 and 100 mg/kg and Gr. VI (piperine 10 mg) alone showed improved MST (%ILS) 22.5 (9.7), 26 (26.8) and 22 (7.3) days,

respectively. The combined groups VII & VIII (curcumin 50 and 100 mg/kg and piperine 10 mg/kg) showed improved MST (%ILS) 27.5 (34.1) and 34.5 (68.2) days, respectively. The standard 5-FU treatment group had highly improved MST (%ILS) up to 37.5 (78.0) days.

The tumor control group showed a significant (P <0.001) decrease in hematological parameters like RBC, HG and significant (P <0.001) increase in WBC count in comparison with the normal control group. The other treatment groups curcumin 50 mg/kg didn't show any significant improvement in RBC count and HG amounts, though there was significant (P <0.05) decrease in the WBC count. Curcumin 100 mg/kg (Gr. V) was found to have significant (P <0.05, P <0.05 and P <0.01) influence in increasing RBC count, HG amount and decreasing WBC count, respectively. Curcumin 50 mg/kg in combination with piperine 10 mg/kg (Gr. VII) showed significant (P <0.01, P <0.01 and P <0.001) influence in improving the RBC, HG and in decreasing WBC count, respectively. Gr. VII (curcumin 100 mg/kg in combination with piperine 10 mg/kg) also showed more significantly improved amount of RBC & HG (P <0.001) but decreased WBC count. The standard 5-FU group (Gr. III) showed significant (P <0.001) increase in RBC count, HG levels, and significant decrease (P <0.001) in the WBC count.

The effect of curcumin, piperine alone and in combination on % ILS and hematological parameters are summarized in Table 2.

Effect on serum biochemical parameters

The tumor control group was found to have significant (P <0.001) elevated levels of ALT, AST and ALP. In the curcumin 50 mg/kg treatment group (Gr. IV), we observed significant (P<0.01, P<0.01 and P<0.05) reduction in ALT, AST and ALP, respectively. Similarly, Gr. V (curcumin 100 mg/kg) too, exhibited significant (P <0.001) reduction in ALT, AST and ALP levels in serum. The piperine 10 mg/kg (Gr. VI) had also shown significantly (P <0.01, P< 0.05 and P <0.05) decreased levels of ALT, AST and ALP, respectively. Groups VII & VIII (curcumin 50 and 100 mg/kg in combination with piperine 10 mg/kg and Standard 5-FU (Gr. III) showed more significantly (P <0.001) decreased levels of ALT, AST and ALP compared to the tumor control. The results on serum biochemical parameters are shown in Fig. 2.

Effect on *in vivo* antioxidant studies

The tumor control group (Gr. III) was found to have significant (P <0.001) increase in malondialdehyde (MDA) levels compared to the normal control. Curcumin 50 mg/kg alsone (Gr. IV) didn't show any significant lowering effect on MDA levels whereas, Gr. V (curcumin 100 mg/kg) showed significant (P <0.05) lowering effect on MDA levels. Piperine 10 mg/kg alone (Gr. VI) also showed any significance in lowering of MDA levels, but in combination with curcumin 50 and 100 mg/kg (Gr. VII & VIII) have shown significant (P <0.01, P <0.001) lowering effect on MDA levels, respectively. The standard 5-FU treated group showed significant

Table 2 — Effects on percentage increase in life span (% ILS) and hematological parameters

Groups	MST	% ILS	RBC (Million cells/mm^3)	WBC (Cells/mm^3 × 10^3)	HG (g/dL)
I	>>50	-	5.47±0.69	5.72±0.71	12.80±1.01
II	20.5	-	2.22±0.38***	15.74±1.06***	5.92±1.01***
III	37.5	78.0	4.07±0.30***	7.48±0.79***	10.80±0.93***
IV	22.5	9.7	2.31±0.39^{ns}	14.07±0.87*	6.73±1.33^{ns}
V	26	26.8	3.23±0.20*	13.54±1.03**	7.98±1.50*
VI	22	7.3	2.38±0.40^{ns}	14.07±0.92*	6.89±0.35^{ns}
VII	27.5	34.1	3.35±0.60**	9.21±0.73***	8.34±1.24**
VIII	34.5	68.2	3.94±0.69***	7.89±0.68***	10.34±0.61***

[Results are expressed as Mean ± SD; n=6 in each group; ***significantly different at P <0.001, ** significantly different at P <0.01, * significantly different at P <0.05, ^{ns} not significant]

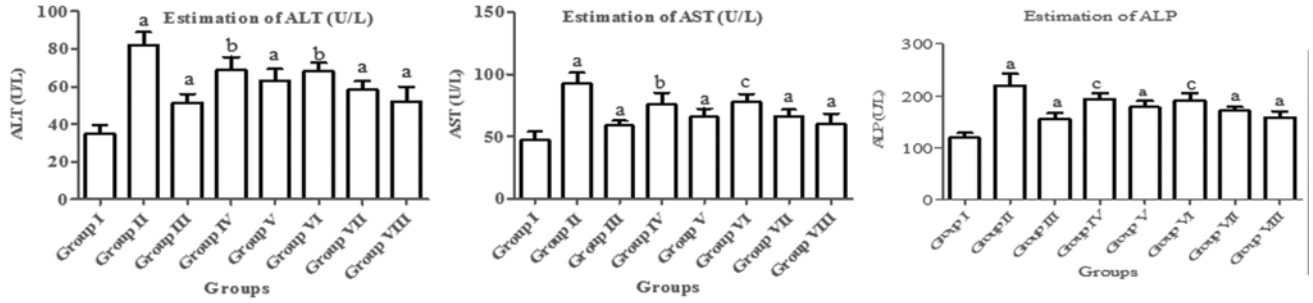

Fig. 2 — Effect on serum biochemical parameters. [Results are expressed as Mean ± SD; n=6 in each group; 'a' 'b' 'c' significantly different at P <0.001, P <0.01 and P<0.05, respectively]

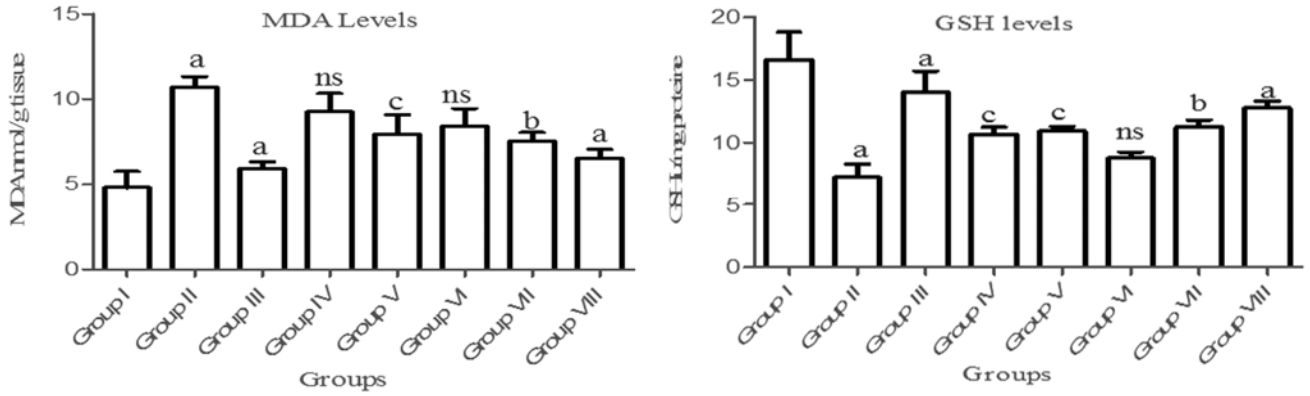

Fig. 3 — Effect on i*n-vivo* anti-oxidant studies. [Results are expressed as Mean ± SD; n=6 in each group; 'a' 'b' 'c' significantly different at *P* <0.001, *P* <0.01 and *P*<0.05, respectively. 'ns' not significant]

(*P* <0.001) lowering effect on MDA levels in comparison with the normal control.

The tumor control (Gr. III) was found to have significant (*P* <0.001) depleted levels of GSH compared to the normal control (Gr. I). Curcumin 50 and 100 mg/kg (Gr. IV & V) were found to have significantly (*P* <0.05) increased levels of GSH. Piperine 10 mg/kg (Gr. VI) didn't show any significant increase in GSH levels, but in combination with curcumin 50 and 100 mg/kg (Gr. VII & VIII) have shown significant (*P*<0.01, *P* <0.001) increase in GSH levels compared to the normal control. The standard 5-FU treated (Gr. III) showed significant (*P* <0.001) increase in GSH levels compared to the normal control. The results on *in vivo* antioxidant studies are depicted in Fig. 3.

Histopathological study

In the histopathological study, we observed normal lobular morphology of the liver without any steatosis, inflammation and infiltration of cells, whereas in the tumor control group steatosis with inflammation and infiltration of cells was obvious. In the curcumin 50 mg/kg (Gr. IV). there was not much difference with tumor control group. However, Gr. V with100 mg/kg curcumin had improved liver morphological structure with comparatively less inflammation. Piperine 10 mg/kg (Gr. VI) didn't show much difference with the tumor control, whereas combination groups (VII & VIIII) 50 and 100 mg/kg curcumin, along with piperine 10 mg/kg have shown normal morphological structure of liver without any steatosis, infiltration of cells and with mild inflammation. The standard 5-FU treatment group was found to have normal morphology without any steatosis, infiltration of cells and with mild inflammation. The histopathological images are shown in Fig. 4.

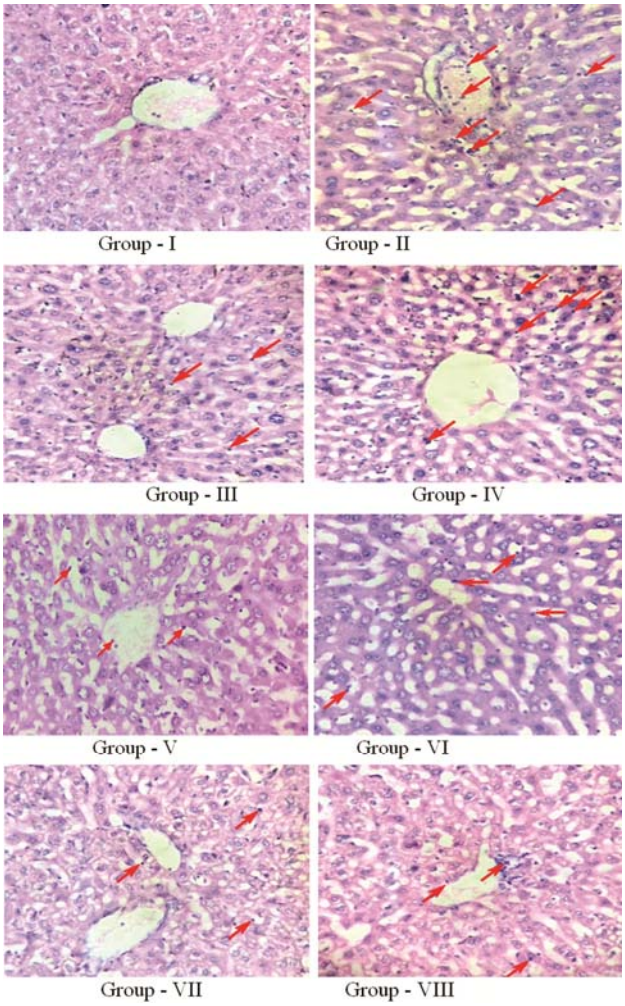

Fig. 4 — Histological alterations in the liver (400X), stained with H&E. [Arrows represents the infiltration of cells]

Discussion

Chemotherapy in cancer treatment often suffers from the issue of resistance towards the cancerous cells and toxicity towards normal cells. Such issues

have encouraged researchers to look for an alternative medicine, particularly from natural sources, that are considered to be safe. In the present study, curcumin has been observed for its anticancer activity alone and in combination with piperine. Curcumin has been reported for many pharmacological actions like Analgesic, antioxidant, anti-inflammatory, anticancer, anticonvulsant, antiasthmatic, antifungal, antimicrobial, antinociceptive, antiarthritic, antiobesity activities[11,13,16,34,35]; amelioration of hepato- and immunotoxicity[15] and also used for cardiovascular[36], metabolic disorders[37]. Despite having many potential health benefits, the clinical application of curcumin is limited due to its low water solubility and poor bioavailability[38]. Here, we tried tried with a natural bioavailability enhancing agent like piperine to improve the bioavailability and its pharmacological action of curcumin as shown in earlier similar works[22,39].

Piperine is a natural compound obtained from black pepper which has been used as a natural bioavailability enhancer; this action has been attributed by its inhibitory effect on hepatic and intestinal glucuronidation of curcumin[40]. Hence, in the present study, we hypothesized that curcumin in the presence of piperine would improve its anticancer potential on DLA lymphoma-bearing mice. Piperine itself has been proved for many pharmacological actions like anti-inflammatory, antinociceptive, antiarthritic, antidepressant, antioxidant and anticancer activities[41].

In the present study, we observed a significant increase in the ascites fluid, in tumor control mice (Gr. II) compared to the normal control group, ascites fluid is the nutritional source for the growth of cancer cells. Along with the DLA cancer cells, ascites fluid also increased in amount, thereby increasing the body wt. of the animals. It is a proper indicator for cancer progress[42]. In the treatment groups, the 5-FU treated group (Gr. III) was found to have significant influence in decreasing DLA cell viability, tumor volume and also the body wt. of the animals. The treatment with curcumin decreased the ascites fluid volume along with the decreased viability of DAL cancer cells. Therefore, the weights of the DLA induced animals were reduced, which demonstrates the anti-proliferative effect of curcumin. Piperine 10 mg/kg alone (Gr. VI) had shown significant decrease in DLA cell viability, tumor volume and body wt. of the animals. Studies have shown that the antitumor activity of piperine in DLA induced solid tumors and EAC induced ascites tumors in animal models[43]. The present results are in alignment with the previous report. The combination of curcumin and piperine was found to have a more significant influence in decreasing in DLA cell viability, tumor volume and the animal weights than alone treated groups.

Another reliable criterion for anticancer activity is prolongation of the lifespan of the animals. The curcumin and pipeline alone treated groups were found to have %ILS comparable to that of the combination groups (Gr. VII & VIII) than the individual treatment groups (Gr. IV-VI) which have shown equipotency with the standard treatment group. Therefore, the %ILS in the combination treatment group, could be attributed to its prevention of tumor development by the combined action of curcumin and piperine.

The previous scientific reports have shown that the myelosuppression and anemia are associated with ascites carcinoma[41]. In the present study, the DLA control group of animals showed reduced RBC, hemoglobin content and elevated WBC count in the animals. The results are in agreement with the previous scientific reports[44]. The anemia observed in DLA bearing mice may be attributed to the reduction in RBC count or iron deficiency, either by hemolysis or myelopathic conditions[45]. In the present study, the combination treatment groups (Gr. VII & VIII) were found to have the better therapeutic benefit of increasing the RBC, hemoglobin contents and also in decreasing WBC count, than the alone treatment groups (Gr. IV-VI). Phytochemical compounds are known to reduce myelotoxicity due to immune boosting and free radical scavenging activity[46]. The curcumin and piperine both have been proven for their antioxidant potential and immune boosting properties in the animal models[13,15,42,47]. Thus, the present results suggest that combination of curcumin and piperine significantly ameliorated the hematological alterations induced by DLA lymphoma.

The ALT, AST, and ALP are the biomarkers which indicate liver function. The elevation of ALT and AST levels are indicative of liver parenchymal cell injury and the elevation of ALP is indicative of cholestasis or infiltrative liver diseases[26]. Impaired liver function was also observed due to cancer and it will be indicated by the elevated levels of ALT, AST and ALP in the serum[48]. In the present study, the

tumor control group was found to have a greater increase in ALT, AST and ALP levels as in line with the previous literature[48]. Earlier reports have also shown that the curcumin and piperin treatment reduce the elevated levels of ALT, AST and ALP by protecting the liver cells from injury[23,49]. In agreement with the previous studies, the individual treatment groups (Gr. IV-VI) were found to have lowered levels of ALT, AST and ALP. In the combination group (Gr. VII & VIII) a greater decrease was obvious in these liver biomarkers, indicating a better therapeutic benefit with the combined effect of curcumin and piperine. The antioxidant potential of both the agents could be attributed for significant decrease in the levels of ALT, AST and ALP.

Tumors itself have the ability to produce more amount of oxygen free radicals which act on the lipid membranes and cause lipid peroxidation producing MAD. The innate antioxidant defensive system mitigates the effects of this oxygen-free radicals[50]. In our study, the tumor control was found to have a significant increase in MDA levels and decreased levels of GSH whereas the treatment groups with curcumin, piperine alone have shown little decrease in MDA and slight improvement in GSH levels. However, in curcumin, along with piperine treatment groups (Gr. VII & VIII), the levels of MDA and GSH were found to be normalized compared to the normal control group of animals. The histopathological investigations have also supported the therapeutic benefit of curcumin and piperine combination for the cancer treatment.

Conclusion

The results of this study suggest that the therapeutic benefits of the curcumin (@ 50 and 100 mg/kg, p.o.) in the treatment of cancer could be improved effectively with the combination of piperine @10 mg/kg, p.o. However, further research is required to understand the mechanism at the molecular level and also the pharmacokinetics of the individual and combination treatment groups.

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Conflict of interest

The authors declare no conflict of interests.

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