

EFFECT OF CURCUMIN AND NANOCURCUMIN ON HEAMATO BIOCHEMICAL PARAMETERS IN DALTON'S ASCITES LYMPHOMA IN MICE

P. Krishnaveni¹, M. Thangapandiyar^{*2}, P. Raja³ and G.V.S Rao⁴

Department of Veterinary Pathology
Madras Veterinary College
Tamil Nadu Veterinary and Animal Sciences University
Chennai, Tamil Nadu, India

ABSTRACT

Curcumin derived from *curcuma longa* has been reported for its anti-cancer activity, but low bioavailability limits the therapeutic potential of curcumin. In this study curcumin and solid lipid nanocurcumin were tested for its therapeutic use and methotrexate was used as the standard drug. Methotrexate at a dose rate of 3.5mg/kg bodyweight and curcumin and nanocurcumin at a dose rate of 100 and 50 mg/kg bodyweight were evaluated in Dalton's Ascites Lymphoma-bearing mice by assessing various hematobiochemical parameters. Hematological parameters were estimated using auto hematology analyzer and biochemical parameters with automatic serum biochemical analyzer. Dalton's Ascites Lymphoma-bearing mice showed reduction in RBC count and amount of Hb and increased leukocyte count with neutrophilia in comparison with normal control group. The tumor control group also showed elevated levels of ALT, AST and ALP while albumin and total protein levels decreased in serum. Treatment with curcumin and nanocurcumin showed significant increase in RBC and Hb count as well as decrease in WBC count. There was a significant decrease ALT, AST and ALP and increase in albumin and total protein levels. Precisely curcumin and nanocurcumin showed significant influence in the restoration of various hematobiochemical parameters.

Keywords: Curcumin, Dalton's Ascites Lymphoma, Solid lipid nanocurcumin

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* Corresponding author; email: sugigold@gmail.com

¹ Postgraduate scholar

² Assistant Professor, Veterinary Clinical Complex,
Veterinary College and Research Institute, TANUVAS
Udumalpet, Tamil Nadu, India

³ Assistant Professor, Department of Animal
Biotechnology

⁴ Professor and Head

INTRODUCTION

Cancer characterized by the uncontrolled growth and spread of abnormal cells is the second most leading cause of death worldwide. The present study mainly focuses on Lymphoma which constitutes around 80

% of the hemopoietic malignancies. Non-Hodgkin's Lymphoma constitute 5,44,000 new cases and 2,60,000 deaths worldwide in 2020 (Sung *et al.*, 2021). Even though we have different strategies for the treatment of cancer like radiation therapy, endocrine therapy, immunotherapy and chemotherapy, they are mostly associated with complications like bone marrow suppression, organ toxicity, alopecia and drug induced cancers Lymphoma is one the most chemotherapy responsive cancer. The limitations with respect to chemotherapy is its non-selectivity and multidrug resistance. Herbal extracts with less side effects and good therapeutic potential have gained lot of attention globally. Curcumin derived from the *Curcuma longa* is known for its anti-inflammatory, antiseptic, antioxidant and anticancerous property. The pharmaceutical role of curcumin is limited due to low water solubility, rapid systemic elimination, inadequate tissue absorption and reduced bioavailability. Nanoparticles enhance the oral bioavailability of the drugs. (Shaikh *et al.*, 2009; Mohanty and Sahoo, 2010). Effect on hemopoietic system is one of the reliable criteria for assessing the quality of anticancerous drug (Dhamija *et al.*, 2011; Sakthivel *et al.*, 2012). The present study aimed at evaluating the anticancerous efficiency of curcumin and nanocurcumin in Dalton's Ascites Lymphoma in mice.

MATERIALS AND METHODS

Preparation of solid lipid nanocurcumin

Curcumin (Sigma catalogue no: 1386, >65% assay-10 g) was obtained from M/s Sigma Aldrich Inc., St. Louis, MO, USA and stored at -20 °C. Solid lipid nanocurcumin (SLN) was prepared by single emulsification

solvent evaporation method as per the procedure given by Pooja *et al.* (2015).

Animals

Male BALB/c mice of 20-25g was procured from M/s Biogen Laboratory animal facility, Anekal, Bengaluru, Karnataka, India-562 107. The animals were fed with standard pellet feed and water was given adlibitum. All the animal experiments were conducted according to the guidelines of Institutional Animal Ethics Committee.

Dalton's Ascites Lymphoma (DAL) cell line

Dalton's Ascites Lymphoma cell line was procured from Amala Cancer Research Centre, Amala Nagar, Thrissur, Kerala, India-680 555.

DAL induced tumor studies

Animals were divided in to four groups. All the animals were inoculated with 1×10^6 cells intraperitoneally. Group 1 was tumor control, Group 2 was treated with methotrexate at a dose rate of 3.5 mg/kg bodyweight, group 3 and 4 were treated with curcumin and nanocurcumin at a dose rate of 100 and 50 mg/kg bodyweight per orally respectively. The blood was collected on the 16th day from all the animals. Blood was collected in heparinized vials and analyzed to estimate hematological parameters like hemoglobin (Hb), packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC) and platelet count using auto hematology analyzer (BC-2800Vet, Mindray Medical Instrumentation, China) using ready-made diagnostic reagent kits supplied by M/s Agappe Diagnostics Limited, Kerala,

India. Serum was separated and utilized for estimation of serum biochemical parameters such as total protein (Direct biuret method), albumin, AST, ALT, ALP (IFCC- International federation of clinical chemistry) by automatic serum biochemistry analyzer (A15 Random Access Analyzer, Biosystems, Barcelona, Spain). All the values were expressed as mean \pm SE. The statistical analysis was performed using SPSS software.

RESULT AND DISCUSSION

Inoculation of DAL cells resulted in significant increase in total WBC count, with increased neutrophils and decreased lymphocyte count as compared to the normal control. Amount of Hemoglobin and RBC count also found to be decreasing with increase in PCV in DAL induced mice. No significant difference noticed in monocyte count. The results were similar to the previous reports by Sakthivel *et al.* (2012). They also observed elevated WBC count and reduced Hb count in DAL bearing mice. Myelosuppression and anemia were frequently observed in ascites carcinoma due to iron deficiency produced either by hemolytic or myelopathic conditions which ultimately lead to reduced RBC count. Induction of myelotoxicity in ascites carcinoma models was found to be of immunological significance in order to meet the adverse conditions developed by the introduction of foreign bodies in the blood (Dhamija *et al.*, 2013). Increase in PCV might be due to dehydration caused by severe ascites. Treatment with curcumin and nanocurcumin reduced the ascites results in decrease in dehydration and PCV. Increased WBC count might be associated with the

defense mechanism against the cancer cells (Loganayaki and Manian, 2012; Augustine *et al.*, 2014). Curcumin and nanocurcumin found to reverse these changes and there was a significant increase in Hb and RBC count whereas WBC count decreased significantly in the curcumin and nanocurcumin group and it were comparable with the results produced by standard drug methotrexate (Table I). Danduga *et al.* (2020) reported that curcumin at 100mg/kg caused significant elevation in RBC and Hb count and decrease in WBC count which is similar to our observations. Phytochemicals reduce myelotoxicity due to immune boosting and free radical scavenging activity (Manjula *et al.*, 2010). As the progression of cancer was brought under control by the curcumin and nanocurcumin the leukocyte count came to near normal in curcumin and nanocurcumin group (Danduga *et al.*, 2020). Treatment with curcumin and nanocurcumin maintain normal hematological values indicating its hemopoietic protection activity.

The levels of ALT, AST and ALP were found to be significantly increased in DAL control mice while total protein and albumin content were decreasing. ALT and AST levels found in serum and mostly associated with liver parenchymal cells and levels were increase in acute liver disease. ALP levels increased in large bile duct obstruction, intrahepatic cholestasis and infiltrative liver diseases (Gaze, 2007). Priya *et al.* (2011) reported similar findings in DAL bearing mice. Impaired liver function was also observed in animals with cancer. The treatment group showed decrease in ALT, ALP and AST levels and albumin as well as total protein content found to be increasing. Similar

Table1. Effect of curcumin and nanocurcumin on hematological parameters

Parameters	DAL Control	DAL + Methotrexate	DAL+ Curcumin	DAL+ Nano curcumin	Normal control	F value
Hb(g/ml)	7.10 ^{a±} 0.218	11.87 ^{c±} 0.341	8.93 ^{b±} 0.78	11.79 ^{c±} 0.38	12.51 ^{c±} 0.25	27.330**
PCV (%)	50.133 ^{d±} 0.47	45.833 ^{c±} 0.365	36.3 ^{a±} 0.55	36.5 ^{a±} 0.8854	37.95 ^{b±} 0.3575	213.659**
RBC × (10 ⁶ / μL)	5.14 ^{a±} 0.92	8.6 ^{b±} 0.24	7.89 ^{b±} 0.12	8.09 ^{b±} 0.497	10.59 ^{c±} 10.598	14.340**
WBC × (10 ³ / μL)	27.40 ^{c±} 0.61	17.54 ^{b±} 0.31	16.76 ^{b±} 0.4346	16.65 ^{b±} 0.338	8.433 ^{a±} 0.72847	262.085**
Neutrophils (%)	73.67 ^{c±} 1.145	42.50 ^{b±} 1.1118	45.17 ^{b±} 1.22	44.17 ^{b±} 3.061	31.67 ^{a±} 2.582	181.509**
Lymphocytes (%)	31.67 ^{a±} 1.054	43.17 ^{b±} 3.43	51.00 ^{c±} 3.098	50.00 ^{c±} 3.225	66.83 ^{d±} 1.046	109.402**
Monocytes (%)	2.5 [±] 0.619	2.67 [±] 0.494	4 [±] 0.966	4.83 [±] 0.601	3.67 [±] 0.882	1.734 ^{NS}

Means with different superscript in arrow differ from each other (P<0.05); One-way ANOVA Duncan's test: *- Significant (P<0.05);**- significant (P<0.01); NS-Non-significant

Table 2. Effect of curcumin and nanocurcumin on serum biochemical parameters

Parameters	DAL control	DAL+ Methotrexate	DAL+ Curcumin	DAL+ Nano curcumin	Normal control	F value
ALT(IU/L)	103.73 ^{c±} 1.86	84.50 ^{b±} 1.13	76.30 ^{b±} 1.96	58.71 ^{a±} 0.98	56.15 ^{a±} 6.46	37.296**
AST(IU/L)	167.13 ^{d±} 6.60	115.44 ^{b±} 0.833	125.89 ^{bc±} 2.51	116.13 ^{b±} 2.29	87.35 ^{a±} 0.64	73.901**
Albumin(g/dL)	1.58 ^{a±} 0.08	2.23 ^{b±} 0.09	1.79 ^{a±} 0.192	2.91 ^{c±} 0.16	3.33 ^{c±} 0.18	23.974**
TP(g/dL)	2.83 ^{a±} 0.16	4.68 ^{b±} 0.154	5.03 ^{bc±} 0.082	5.25 ^{c±} 0.098	6.95 ^{d±} 0.138	122.539**
ALP(IU/L)	143.79 ^{c±} 0.64	83.42 ^{a±} 1.077	83.418 ^{a±} 0.077	97.99 ^{b±} 0.76	81.32 ^{a±} 0.456	993.150**

Means with different superscript in arrow differ from each other (P<0.05); One-way ANOVA Duncan's test: *- Significant (P<0.05);**- significant (P<0.01); NS - Non-significant

results were observed by Danduga *et al.*(2020) on treatment with curcumin at a dose rate of 100 mg/kg bodyweight and curcumin piperine combination showed better results than curcumin alone indicating combinations can improve the oral bioavailability of curcumin. The standard drug also produced similar results as that of treatment group (Table II). In the treatment group there was an obvious decrease in the levels of liver biomarker indicating liver protective effect and better therapeutic benefit by curcumin and nanocurcumin. The results of this study indicate that curcumin at 100 mg/kg body weight and nanocurcumin at 50 mg/kg body weight showed better hemopoietic as well as liver protective function. Curcumin incorporated in solid lipid nanoparticles at half the dose of curcumin itself produced better results.

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