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CURCUMINOIDS AMELIORATE HEMATOLOGICAL TOXICITY INDUCED BY ZIDOLAM IN RAT MODEL

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ABSTRACT

Protective role of curcuminoids on zidolam (zidovudine and lamivudine) induced hematological toxicity was evaluated in albino rats. Zidolam at 12.9 mg/kg orally was administered to rats for 21 days to induce hematological toxicity. Hemoglobin (Hb%), hematocrit (Hct), red blood cells count (RBC), RBC indices like mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), bleeding time and clotting time parameters were assessed. *In-vivo* antioxidant parameters, lipid peroxidation, superoxide dismutase (SOD), reduced glutathione (GSH) and catalase were also assessed. Treatment with curcuminoids for 30 days at 100 and 200 mg/kg, orally, protected the animals from hematological toxicity induced by zidolam. Significant effect on bleeding and clotting time was not observed. A significant *in vivo* antioxidant activity was also observed in the curcuminoid treated animals. The results of the present study indicate that curcuminoids possess significant protection against hematological toxicity induced by zidolam.

Key words: Zidolam, Hematological toxicity, Curcuminoids, Protective role

INTRODUCTION

Hematological toxicity is a prominent adverse effect on long term usage of drugs like sodium valproate[1], methotrexate[2], foscarnet, ganciclovir, zidovudine and high doses of cotrimoxazole[3]. These drugs damage bone marrow inhibiting the formation of blood cells leading to anemia and neutropenia. Other factors like age, gender, nutritional deficiencies (vitamin B_{12}, folate and iron), and metal poisoning with lead also contribute for the hematotoxicity[4].

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Zidolam an antiretro viral agent, also known as combivir, consists of zidovudine and lamivudine. Zidolam induced hematotoxicity is a widely used and validated animal model to induce hematological deficits in rat model[5]. In India, research is being focused on the usage of natural remedies as adjuncts to conventional therapy and evaluation of natural products may provide a useful remedy for various drug-induced toxicities. Turmeric is a popular Indian spice derived from the rhizome of *Curcuma longa* (Family: Zingiberaceae). Curcuminoids is a mixture of curcumin, demethoxycurcumin and bisdemethoxycurcumin and considered vital constituents of turmeric[6]. Curcuminoids were reported with pharmacological activities such as antiinflammatory[7], anticancer[8], antioxidant[9] and wound healing properties[10]. In the present study, the protective effects of curcuminoids on Zidolam induced hematological toxicity and their antioxidant activity in albino rats was systematically evaluated.

MATERIALS AND METHODS

Chemicals

Curcuminoids is obtained as a gift sample from Indo Necop Chemical Pvt ltd., Vinukonda. Zidolam was purchased from Genix Pharma Ltd, Hyderabad. Other chemicals used were of analytical grade from EMerck and SD fine.

Animals

Healthy adult male albino rats of Wistar strain were procured from Sri Venkateswara Enterprises, Bangalore. The animals were acclimatized for the laboratory conditions for a period of ten days at room temperature (27±3^{o}C), and relative humidity (65±10%). All animals were fed with standard rodent-pellet diet supplied from VRK Nutritional Solutions Company, Bangalore and water *ad libitum*. The animal experimentation procedures were approved by our Institutional Animal ethical committee (SPMVV/IAEC/2014a/12).

Induction of Hematological toxicity

Zidolam (zidovudine and lamivudine) at a daily dose of 12.9 mg/kg of body weight, orally was administered to rats for a period of 21 days to induce hematological toxicity[5]. The induction of hematological toxicity was confirmed by the estimation of hematological parameters.

Treatment Protocol

Animals were divided in to four groups, each group consisting of six animals. Group I animals left untreated and served as normal control. Group II, III and IV animals were treated with zidolam (12.9mg/kg, orally) for 21 days to induce hematological toxicity. Group- II animals with hematological toxicity were further treated with vehicle 1% carboxy methyl cellulose suspension for 4 weeks. Groups III and IV animals with hematological toxicity were treated with curcuminoids orally at a dose of 100 and 200 mg/kg, respectively for 4 weeks.

Collection of blood samples

In all groups, on day 21, blood was withdrawn from retro orbital venous puncture collected in EDTA coated anticoagulant bottles. In the post-induction groups, blood was withdrawn on day 28, 35, 42 and 49. The blood samples were further used for the analysis of hematological parameters.

Hematological parameters

Hematological parameters such as haemoglobin (Hb %), hematocrit (Hct), red blood cell count (RBC), RBC indices like mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), clotting and bleeding time were estimated by standard methods[11].

In vivo antioxidant studies

At the end of the study on day 49, blood was collected from all the groups and centrifuged for 10 min at 5,000 rpm, plasma was separated, while the RBC were washed three times with 0.9% sodium chloride and the washed erythrocytes were lysed with distilled H_2O (1:3 v/v) at 0^{o} C for 30 minutes and these samples were stored at -80^{o} C before performing the antioxidant studies[12]. The assessment of *in vivo* anti oxidant parameters such as lipid peroxidation[13], superoxide dismutase (SOD)[14], reduced glutathione (GSH)[15] and catalase[16] were carried out.

STATISTICAL ANALYSIS

All the values were expressed as Mean ± S.E.M. The data was analyzed using analysis of variance (ANOVA) followed by Dunnett's multiple comparison test by using software Graph Pad Prism Version 6. In all tests $P < 0.05$ was considered as statistical significance.

RESULTS

Administration of zidolam (12.9 mg/kg) has induced significant hematological toxicity in groups II, III and IV on day 21 as demonstrated by significant decrease in the levels of Hb%, Hct, RBC, MCV, MCH, MCHC and WBC. No significant change in the clotting and bleeding time was observed when compared to normal animals (Table 1). Treatment with curcuminoids in group III and IV animals at 100 and 200 mg/kg doses significantly (P< 0.001) increased Hb%, Hct, RBC, MCV, MCH, MCHC and WBC levels when compared to disease control on day 28, 35, 42 and 49 (Tables 2a,b and c). Administration of curcuminoids did not show any significant difference in bleeding time and clotting time when compared to disease control animals (Table 3).

On treatment with zidolam, a significant (P< 0.001) increase in the lipid peroxidation was observed in the disease-control animals when compared to the normal group, whereas treatment with curcuminoids at both the doses (Group III and IV) has significantly decreased lipid peroxidation levels when compared to the disease control group (II). Other *in vivo* antioxidant enzymes like SOD, GSH, Catalase levels were significantly (P< 0.001) reduced in the disease- control group when compared to normal animals. Treatment with curcuminoids (Group III and IV) significantly (P< 0.001) increased the levels of these enzymes compared to disease- control group attesting the significant antioxidant activity of curcuminoids (Figures 1, 2, 3 and 4).

Table: 1 Effect of zidolam (12.9 mg/kg) on hematological parameters on day 21

Groups	Hemoglobin (Hb %)	Hematocrit (Hct)	Red blood cell count (RBC)	Mean corpuscular volume (MCV)	Mean corpuscular hemoglobin (MCH)	Mean corpuscular hemoglobin concentration (MCHC)	White blood cell count (WBC)	Clotting time	Bleeding time
Control	15.32 ±0.42	44.29 ±0.23	8.96 ±0.42	51.63 ± 1.23	18.09 ±1.09	33.59 ±1.22	13000 ± 872.8	1.51 ±0.12	2.09 ±0.21
Diseased-Control	9.89 ±1.08[a]	25.89 ±0.98[a]	5.95 ±0.31[a]	48.60 ±1.92[b]	16.14 ±1.82[c]	31.92 ±1.93[c]	9432 ±789.2[a]	1.41 ±0.92	1.92 ±0.76
Low dose	9.91 ±0.83 [a]	26.12 ±0.42 [a]	5.81 ±0.09 [a]	46.78 ±1.83[b]	16.62 ±1.34[c]	30.05 ±1.55[b]	9870 ±823.7 [a]	1.48 ± 0.48	1.98 ±0.01
High dose	9.65 ±0.87 [a]	24.12 ±0.21 [a]	5.67 ±0.82 [a]	47.89 ±1.37[b]	16.89 ±1.82[c]	30.77 ±1.22[b]	9832 ± 864.1 [a]	1.44 ±0.41	1.95 ±0.71

a- P<0.001, b- P<0.01 and c -p<0.05 compared to control group, Ns- No significant

Table 2a: Effect of curcuminoids on Haemoglobin (Hb %), Hematocrit (Hct) and Red blood cells count (RBC).

Groups	Hb (g/dl)				Hematocrit (%)				RBC (x 10^6/ mm^3)			
	28	35	42	49	28	35	42	49	28	35	42	49
Control	16.08 ±0.58	16.10 ±0.385	16.15 ±0.385	16.20 ±0.278	46.69 ±0.87	48.78 ±0.78	49.18 ±0.65	49.87 ± 0.98	8.795 ±0.385	8.795 ±0.385	8.835 ±0.285	8.858 ±0.31
Disease control	9.30 ±1.11[a]	9.197 ±0.170[a]	9.142 ±0.710 [a]	9.075 ±0.69 [a]	26.86 ±1.23[a]	27.89 ±1.09[a]	28.05 ±1.72[a]	29.18 ±1.60[a]	5.205 ±0.170[a]	5.855 ±0.61[a]	5.843 ±0.61[a]	5.802 ±0.70 [a]
Low dose	10.24 ±0.68[b+]	10.86 ±1.66 [b+]	11.21 ±0.008[a+]	12.08 ±0.416 [a+]	30.72 ±0.97[b+]	31.34 ±0.78[b+]	32.98 ±0.42[a+]	34.78 ±0.82[a+]	6.100 ±0.167[b+]	6.697 ±0.01[b+]	7.363 ±0.178[a+]	7.043 ±0.470[a+]
High dose	11.10 ±0.76[b+]	12.33 ±1.136[b+]	12.68 ±1.45 [a+]	14.15 ±0.6411[a+]	33.89 ±0.74[b+]	34.56 ±0.67[b+]	34.78 ±0.43[a+]	35.18 ±0.42[a+]	6.617 ±0.183[b+]	6.832 ±0.021[b+]	7.672 ±0.109[a+]	7.982 ±0.294[a+]

Table 2 b: Effect of curcuminoids on Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration

Groups	MCV (femtoliter)				MCH (picogram)				MCHC (g/dl)			
	28	35	42	49	28	35	42	49	28	35	42	49
Control	53.11 ±2.01	52.46 ±1.39	51.66 ±1.62	52.29 ±1.42	18.28 ±1.23	18.51 ±2.91	18.27 ±2.12	18.28 ±1.90	34.43 ±1.92	33.00 ±1.32	33.83 ±1.47	33.48 ±1.37
Disease control	48.60 ±1.23^{c}	48.63 ±1.02^{c}	47.00 ±1.81^{c}	47.29 ±1.93^{b}	15.64 ±1.77^{c}	15.70 ±2.11^{c}	15.84 ±1.09^{c}	17.86 ±1.72^{c}	31.62 ±1.31^{b+}	31.97 ±1.87^{c+}	32.59 ±1.34^{c+}	31.10 ±1.23^{c+}
Low dose	45.79 ±1.82^{b+}	46.79 ±1.70^{c+}	49.38 ±1.90^{c+}	50.36 ±1.46^{c+}	16.24 ±1.93^{c+}	17.21 ±2.31^{c+}	18.80 ±1.92^{c+}	19.51 ±1.42^{c+}	32.33 ±1.45^{c+}	34.65 ±1.93^{b+}	34.99 ±1.22^{b+}	34.73 ±1.57^{b+}
High dose	45.07 ±1.55^{b+}	45.33 ±1.52^{b+}	50.58 ±1.02^{a+}	51.21 ±1.76^{a+}	17.57 ±1.62^{c+}	18.74 ±2.10^{b+}	19.27 ±1.32^{b+}	20.04 ±1.23^{b+}	32.75 ±1.56^{c+}	35.67 ±1.85^{b+}	36.45 ±1.67^{a+}	36.22 ±1.30^{a+}

Table 2c: Effect of curcuminoids on WBC count

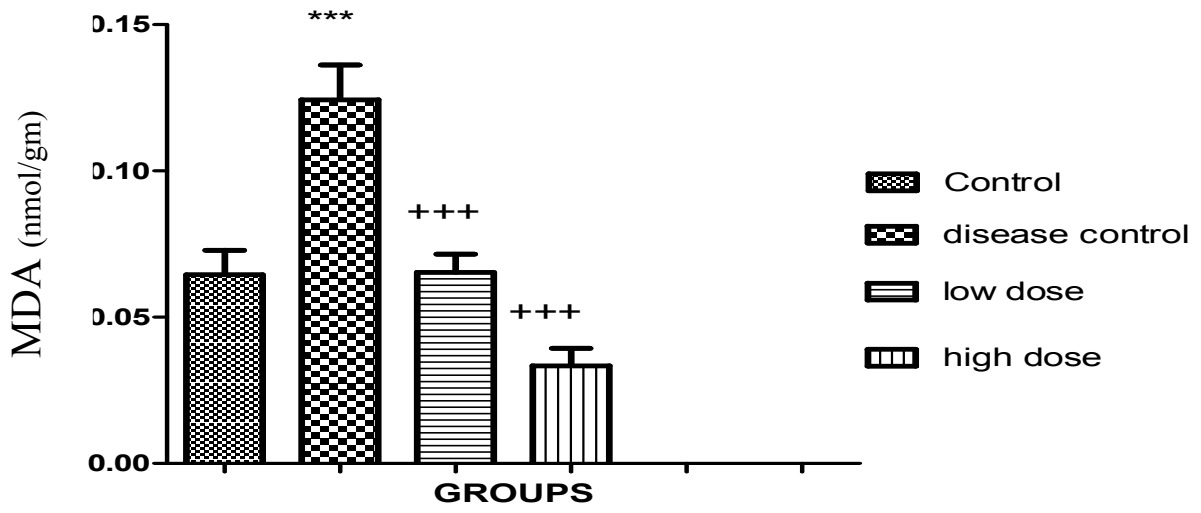
Groups	WBC (x 10^3/mm^3)			
	28	35	42	49
Control	13000 ±632.5	13000 ±632.5	13000 ±632.5	13000 ±632.5
Disease control	8525 ±402.2^{a}	8492 ±411.0 ^{a}	8447 ±402.2^{a}	8413 ±402.1 ^{a}
Low dose	8608 ±485.2^{Ns}	8933 ±398.3^{Ns}	9400 ±481.7^{b+}	11375 ±976.1^{a+}
High dose	8817 ±421.5^{Ns}	9282 ±495.6^{c+}	9650 ±441.6^{a+}	11833 ±811.0^{a+}

Table 3: Effect of curcuminoids on clotting time and bleeding time

Groups	Clotting time (Minutes)				Bleeding time (Minutes)			
	28	35	42	49	28	35	42	49
Control	1.607 ±0.34	1.66 ±0.33	1.607 ±0.34	1.66 ±0.33	2.08 ±0.15	2.03 ±0.18	2.80 ±0.15	2.26 ±0.18
Disease control	1.66 ±0.33^{Ns}	1.76 ±0.41^{Ns}	1.66 ±0.33^{Ns}	1.76 ±0.34^{Ns}	2.03 ±0.158^{Ns}	1.01 ±0.21^{Ns}	2.30 ±0.158^{Ns}	1.99 ±0.21^{Ns}
Low dose	1.68 ±0.34^{Ns}	1.96 ±0.04^{Ns}	1.18 ±0.34^{Ns}	1.69 ±0.11^{Ns}	1.99 ±0.21^{Ns}	1.98 ±0.20^{Ns}	1.90 ±0.20^{Ns}	1.79 ±0.02^{Ns}
High dose	1.69 ±0.40^{Ns}	1.66 ±0.30^{Ns}	1.60 ±0.34^{Ns}	1.66 ±0.13^{Ns}	1.98± 0.20^{Ns}	2.85 ±0.15^{Ns}	1.98 ±0.02^{Ns}	2.96 ±0.15^{Ns}

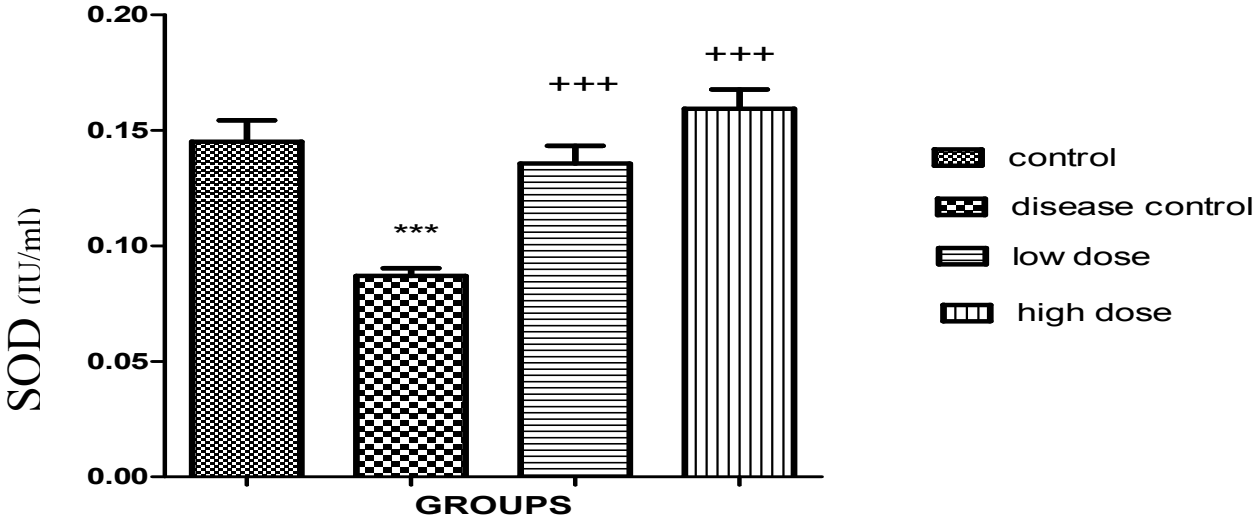
b- P<0.001, b- P<0.01 and c -p<0.05 compared to control group, a+ - P<0.001, b^{+}- P<0.01 and c^{+} -p<0.05 compared to disease control group, Ns- No significant

Figure 1: Effect of curcuminoids on MDA levels



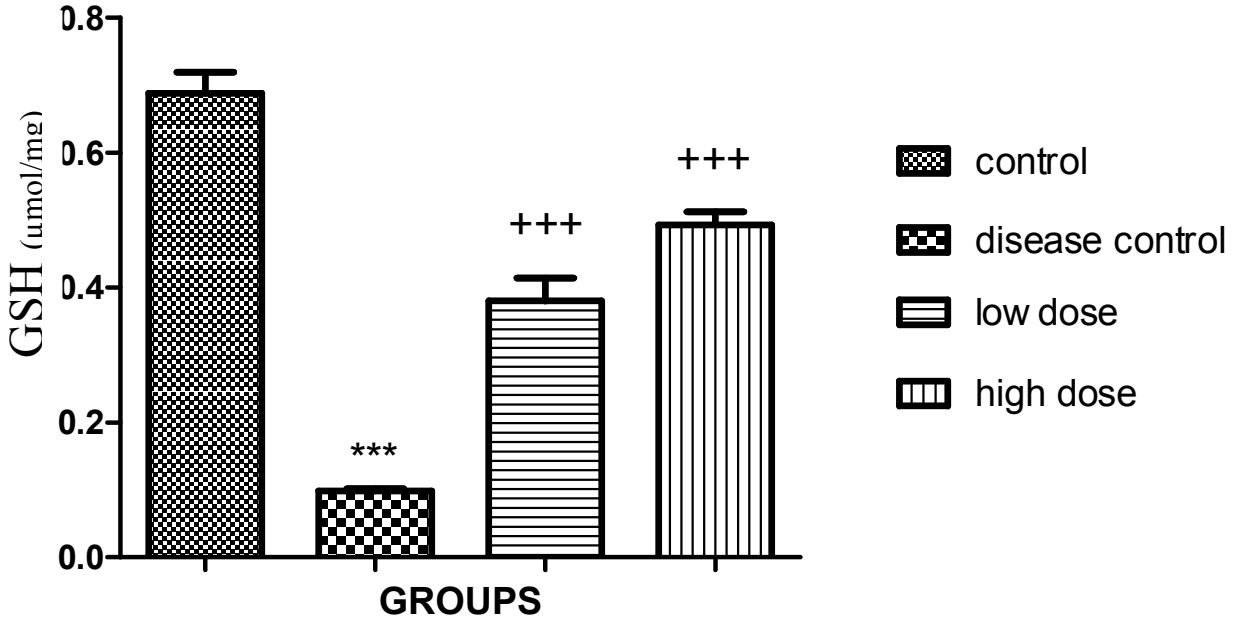
***-p<0.001 control compared to control, +++-p<0.001 compared to disease control

Figure 2: Effect of curcuminoids on SOD levels



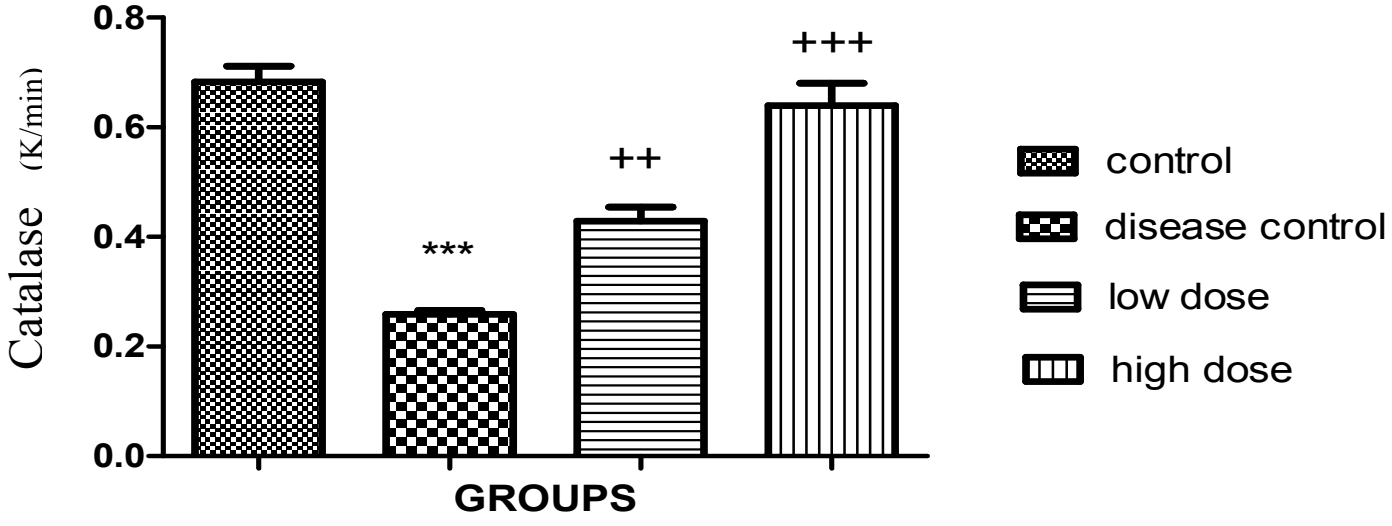
***-p<0.001 control compared to control, +++-p<0.001 compared to disease control

Figure 3: Effect of curcuminoids on GSH levels



***-p<0.001 control compared to control, +++-p<0.001 compared to disease control

Figure 4: Effect of curcuminoids on Catalase levels



***-p<0.001 control compared to control, +++-p<0.001 compared to disease control

DISCUSSION

In the present study, zidolam induced hematological toxicity is controlled by curcuminoids and is probably mediated by an enhanced stimulation of erythropoiesis and synthesis of progenitor cells of the bone marrow. This observation is compatible with the earlier studies done on aqueous ethanolic extract of *Mangifera indica* stem bark against cisplatin induced hematological parameters in albino rats[17], protective effect of *Withania somnifera* root extract against led-nitrate induced hematological toxicity[18], aqueous extract of *Passiflora edulis* on biochemical and hematological parameters of Wistar albino rats[19], toxicological study of *Ocimum sanctum Linn* leaves on hematological profile[20]. Zidolam affects hematopoietic system by inhibition of Delta-aminolevulinic acid dehydratase (ALAD), a cytosolic sulfydryl enzyme and ferrochelatase thereby inhibition of heme and hemoglobin synthesis that ultimately led to anemia[21]. The protective effects of curcuminoids on RBCs can be attributed to their ability in restoring the levels of thiol antioxidants eg, N-acetyl cystine (NAC) as well as by acting as chelating agent, thus reducing further damage of RBC[22]. Curcuminoids decreases the toxic effect of zidolam treated groups thus indicating the protective role of curcuminoids on anemic conditions. In our study RBC indices like MCV and MCH levels low in zidolam induced groups when compared to normal group, refers microcytic and hypochromic anemia. This was slowly improved after treatment with curcuminoids.

WBC levels were significantly decreased with zidolam treatment in groups II, III and IV and this may be due to inhibition of progenitor cells in the bone marrow. In our study curcuminoids offered protection against zidolam induced leucopenia, this may be due to stimulation of immune system[23]. But, no significant alterations were observed in bleeding and clotting time in all the studied groups. This indicates that zidolam may not interfere with synthesis and functions of proteins involved in clotting cascade. Oxidative stress has been reported to play an important role in the genesis of hematological toxicity. Oxidative stress is a shift reaction between oxidation-reduction that means redox reaction, which leads to cellular damage and is indicated by oxidized products of lipids and proteins. Effects of reactive oxygen species (ROS) generation have been postulated to be major contributors to hematological toxicity induced by Zidolam[5]. This effect can be related to the reduction in specific antioxidant mechanisms, such as decreased GSH, SOD and CAT levels. Curcuminoids represents an excellent class of antioxidants[24.] Curcuminoids are potent scavengers of a variety of reactive oxygen species (ROS) including superoxide anion (O_2^-) and hydroxyl radicals (OH^-)[25]. Similar effect is also observed in our study as noted by enhanced levels of SOD, GSH, catalase conforming the scavenging of ROS free radicals generated by zidolam. This might be responsible for rapid normalization of hematological parameters and decreased levels of lipid peroxidation in groups treated with curcuminoids. Our study clearly indicated that curcuminoids has a potential role against the hematological toxicity induced by zidolam.

CONCLUSION

In our study, curcuminoids has offered protection against zidolam induced hematological toxicity and oxidative stress in rats. Further studies to explore the mechanism of action of curcuminoids on hematopoiesis will be fruitful.

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