Biochemical Effect of Cholchicine on Experimental Leukemia in Rats

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A B S T R A C T

Colchicine is an alkaloid that has been widely used for treatment of gout. It also has a curative effect on cancer, as many studies have shown that its effect on cell apoptosis .The objective of the present study was to evaluate the Biochemical Effect of Cholchicine on Experimentally Induced Leukemia in Rats. Sixty white albino male rats of 8-10 weeks old and 150-200 g weight were ued in the experiment. Rats were randomly divided into six groups 1st group act as normal control , 2nd group was injected intraperitoneally with colchicine (0.14mg/kg/wt) one dose daily for four weeks and act as positive control , 3rd group was administrated orally with benzene (1mg /kg /wt) one dose daily four weeks for induction of leukemia and act as leukemic group , last three groups (4th , 5th ,6th) administrated benzene at a dose of (1mg /kg /wt) daily and treated with colchicine with different concentration (0.07mg/kg/wt) (0.14mg/kg/wt) and (0.21mg/kg/wt) respectively. Blood samples were collected twice after 2 and 4 weeks for biochemical examination. Intraperitoneal injection of colchicine caused increased in Hb level, platelets count and Caspase- 3 and decreased in Total leucocyte count (WBCs),L.malondialdehyde (L-MDA), Super Oxide Dismutase (SOD) ,Catalase (CAT), Glutathione peroxidase (GPx) , Glutathione (GSH),Alpha –fetoprotein (AFP) , Interleukin-2 (IL-2), Interleukin-6 (IL-6), Tumor necrosis factor (TNFα), compared with diseased group.

Key words: Leukemia, Benzene, Colchicine, TNFα, IL-6.

(http://www.bvmj.bu.edu.eg) **(BVMJ-35(1): 84-93, 2018)**

1. INTRODUCTION

Leukemia was defined as increase propagation of blood cells in the bone marrow that increased level of abnormal white blood cells with not completely developed called as blasts or leukemia cells, leukemia widespread in children and adults (Burhan 2016)it is common malignancy in childhood also the acute leukemia is a malignant disorder of white cells caused by a failure of normal differentiation of haemopoietic stem cells and progenitors into mature cells (Kumar al., 2014).

Leukemia results from a mutation in a single stem cell, the progeny of which form a clone of leukaemic cells. Often there is a series of genetic alteration rather than a single event. Genetic events contributing to malignant transformation include inappropriate expression of oncogenes and loss of function of cancer-suppressing genes. Acute leukemia is a condition produced by an abnormal expression of genes, which is generally a result of chromosomal translocation (Mehdi et al., 2015).

The natural products, especially microtubule-binding natural products, as colchicine play important roles in the war against cancer (Yue et al., 2010) where is

microtubules are highly dynamic cytoskeletal fibers composed of α- and β-tubulin heterodimers, and are involved in a variety of fundamental cellular processes, such as the maintenance of cell shape, intracellular trafficking, cell movement, and most recognized mitosis, where most microtubule-binding agents induce apoptosis by the intrinsic mitochondrial-mediated pathway (Yan et al., 2016) .

Benzene causes acute myeloid leukemia and probably other hematological malignancies. As benzene also causes hematotoxicity even in workers exposed to levels below the US permissible occupational exposure limit of 1 part per million, further assessment of the health risks associated with its exposure, particularly at low levels, is needed (McHale et al., 2012).

Colchicine, a natural product of Colchicum autumnae currently used for gout treatment, is a tubulin targeting compound which inhibits microtubule formation by targeting fast dividing cells. This tubulin-targeting property has lead researchers to investigate the potential of colchicine and analogs as possible cancer therapies (Larocque et al., 2014) .

Colchicine has been reported to play important roles in hepatoprotection, anti-inflammation in vitro anti-cancer activity (Hussein and Boshra 2013), where (Lin et al., 2013) showed that colchicine-treated mice had lower increased tumor volume ratios, slower tumor growth rates and larger percentages of tumor necrotic areas than control mice.

Aim of the present study was designed to evaluate possible protective and therapeutic effect of colchicine administration on leukemia in rats.

2. MATERIALS AND METHODS

2.1. Rats and experimental design

Sixty white albino male rats of 8-10 weeks old and 150-200 g weight were were obtained from the laboratory animal research center , faculty of veterinary medicine ,Moshtohor, Benha university. Rats were housed in metal cages ; Fresh and clean drinking water was supplied. All animals were left for acclimatization before the beginning of experiment. Rats were randomly divided into six equal groups 1st group act as normal control , 2nd group was injected intraperitoneally with colchicine (0.14mg/kg/w) one dose daily for four weeks and act as positive control , 3rd group was administrated orally with benzene (1mg /kg /w) one dose daily four weeks for induction of leukemia and act as diseased group , The last three groups (4th , 5th ,6th) were administrated orally with benzene (1mg /kg /w) one dose daily for induction of leukemia and treated with colchicine with different concentration (0.07mg/kg/wt) , (0.14mg/kg/wt) and (0.21mg/kg/wt) respectively.

2.2. Drugs

- Colchicine powder extract from plants of genus colchicum commercially obtained from El Nasr Pharmaceutical Chemicals Company, Cairo .Colchicine was given for albino male rats by (Intraperitoneal Injection) single dose for 30 days where colchicine prepared in (0.9% saline)

- Benzene were obtained from El Gomhouria for Chemicals and Medical Appliance Company, Tanta, El Gharbia, which administrated orally at a dose level (1 mg /Kgb.wt).

2.3. Blood Samples and parameters

Blood samples were collected twice after 2 and 4 weeks from the onset of treatment from retro-orbital plexus of eye of all group.

Blood Samples were divided into two groups :

-The first part using EDTA for hematological parameters: hemoglobin (Hb) (Van Kampen and Zijlstra, 1961), Total leucocyte count (WBCs)(Wintrobe 1981) , Platelets count (Eggleton and Sharp 1963).

-The second part was centrifuged to separate serum for biochemical parameters ,L.malondialdehyde (L-MDA) (Ohkawa,etal.,1979), Super Oxide Dismutase (SOD) (Marklund and Marklund, 1974) ,Catalase (CAT) (Aebi,1984), Glutathione peroxidase (GPx) (Paglia and Valentine, 1967) , Glutathione (GSH) (Beutler et .,1963),Alpha –fetoprotein (AFP) (Engvall et al.,1980), Interleukin-2 (IL-2) and Interleukin-6 (IL-6) (Robb,1984) Tumor necrosis factor (TNFα) (Beyaert and Fiers 1998), Caspase 3(Kumar, 1995).

2.4. Statistical analysis

The results were expressed as mean (±S.E.)and statistical significance was evaluated by one way ANOVA using SPSS (version 10.0) program followed by the post hoc test ,least significant difference (LSD).Values were considered statistically significant when $p<0.05$. (Snedecor,1989).

3. RESULTS

The obtained data in table (1) showed that rats which administrated benzene only (3^{rd} group) expressed significant increase in WBCs count and significant decreases in Hb level and platelets number compared to control groups, where leukemic rats groups that treated to colchicine caused increases in Hb and platelets number and decreases in WBCs count compared with diseased group.

The obtained data in table (2) showed that rats that administrated benzene solely (3rd group, diseased) expressed significant decreases in enzyme , Glutathione , SOD , GPx and an increase in MDA compared to control groups , wherever leukemic rats groups that treated with colchicine with different concentration caused significant decreases in enzyme (CAT), (GSH), (SOD) , (GPx) and (MDA) compared to benzene group (diseased) because the lowest value with the highest concentration of colchicine (0.21mg/kg/wt, 6th group).

The obtained data in table (3) showed that rats which administrated benzene only (3^{rd} group) showed significant increase in plasma (IL-2), (IL-6), (AFP) and (TNFα) compared to control groups where leukemic rats groups that treated with colchicine caused significant decrease in IL-2, IL-6, AFP, TNFα compared to diseased group as the lowest value with the highest concentration of colchicine (0.21mg/kg/wt ,6^{th} group). Table (6) also showed that mean of caspase-3 level significantly decreases after administration of benzene only and induction of leukemia and after treatment with colchicine there are a significant increases in mean of caspase-3 is observed after 2 and 4 weeks compared to benzene group as the highest value with the highest concentration of colchicine (0.21mg/kg/wt, 6th group).

Table (1) Effect of colchicine on WBCs, Hb and platelets in leukemic which induced by benzene .

Blood Parameter	WBCs		Hb		Platelets	
Control 1st	5.84±0.25^{e}		13.59±0.57^{a}		362.0±14.56^{a}	
	15/day	30 /day	15/day	30 /day	15/day	30 /day
Colchicine 2nd	6.56±0.28^{e}	6.62±0.28^{e}	13.51±0.57^{a}	13.07±0.55^{ab}	338.0±14.16^{b}	323.8±13.58^{b}
Diseased 3rd	16.36±0.72^{b}	21.05±0.88^{a}	12.22±0.51^{b}	11.54±0.48^{cB}	309.2±12.92^{c}	209.4±8.73^{d}
Col.(0.07mg) + benzene 4th	18.96±0.80^{a}	15.29±0.64b	11.07±0.47^{c}	11.85±0.50^{cA}	221.2±9.29^{d}	234.0±9.84^{c}
Col.(0.14mg) + benzene 5th	14.99±0.63^{c}	13.16±0.55^{c}	11.86±0.50^{b}	12.21±0.51^{bc}	198.2±8.16^{e}	218.4±9.17^{cd}
Col.(0.21mg) + benzene 6th	13.97±0.59^{d}	9.00±0.38^{d}	12.71±0.53^{ab}	13.38±0.56^{a}	200.2±8.60d^{e}	217.2±9.04^{cd}

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

Table (2) Effect of colchicine on Catalase , Glutathione ,Superoxide dismutase , Glutathione peroxidase and Malondialdehyde in leukemic rats which induced by benzene .

Blood Parameter	Catalase		Glutathione		Superoxide dismutase		Glutathione peroxidase		Malondialdehyde	
Control 1st	36.28±1.97^{b}		70.79±2.02^{b}		36.50±0.51^{b}		36.09±1.74^{b}		54.92±2.71^{e}	
	15/day	30/day	15/day	30 /day	15/day	30 /day	15/day	30/day	15/day	30/day
Colchicine 2nd	38.93±0.40^{a}	79.41±2.28^{a}	79.41±2.28^{a}	76.87±4.00^{a}	39.47±0.50^{a}	41.64±0.43^{a}	38.69±3.21^{a}	41.60±2.47^{a}	63.50±2.94^{e}	58.54±5.53^{e}
Diseased 3rd	12.38±0.43^{c}	53.66±3.48^{c}	53.66±3.48^{c}	40.23±0.57^{c}	17.38±0.32^{c}	16.27±0.95^{c}	16.26±2.42^{c}	16.42±0.39^{c}	150.96±5.46^{a}	187.85±3.46^{a}
Col.(0.07mg) + benzene 4th	10.52±0.37^{cd}	45.61±2.96^{d}	45.61±2.96^{d}	34.19±0.48^{d}	14.77±0.27^{d}	13.83±0.81^{d}	13.82±2.06^{c}	13.95±0.33^{c}	129.74±5.93^{b}	159.67±2.94^{b}
Col.(0.14mg) + benzene 5th	8.91±0.31^{d}	38.63±2.51^{e}	38.63±2.51^{e}	28.96±0.41^{e}	12.50±0.23^{e}	11.71±0.68^{e}	11.71±1.74^{d}	11.81±0.28^{d}	108.81±3.84^{c}	135.24±2.48^{c}
Col.(0.21mg) + benzene 6th	8.04±0.28^{d}	34.87±2.26^{e}	34.87±2.26^{e}	26.15±0.37^{e}	11.29±0.21^{f}	10.78±0.51^{f}	10.57±1.58^{d}	10.67±0.25^{d}	98.23±3.47^{d}	122.1±2.25^{d}

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter

Table (3) Effect of colchicine on Interlukein-2, Interleukein-6, Alpha-Fetoprotien, Tumor necrosis factor α and caspase-3 in leukemic rats which induced by benzene .

Blood Parameter	Interlukein-2		Interleukein-6		Alpha-Fetoprotien		Tumor necrosis factor α		Caspase-3	
Control 1st	3.84±0.50^{c}		9.05±0.77^{c}		4.02±0.41^{d}		25.00±1.68^{e}		0.452±0.01^{d}	
	15/day	30 /day	15/day	30 /day	15/day	30 /day	15/day	30/day	15/day	30/day
Colchicine 2nd	3.31±0.47^{d}	5.22±0.65^{d}	8.65±0.54^{d}	11.47±1.17^{d}	3.73±0.28^{c}	5.20±0.63^{e}	26.94±2.65^{d}	31.18±1.27^{e}	0.436±0.02^{d}	0.43٥±0.02^{d}
Diseased 3rd	12.76±0.88^{a}	18.11±1.77^{a}	38.08±6.33^{a}	82.05±6.43^{a}	8.90±0.49^{a}	11.20±0.6^{a}	91.20±5.38^{a}	136.1±9.19^{a}	0.310±0.01^{e}	0.31٢±0.01^{e}
Col.(0.07mg) + benzene 4th	10.84±0.75^{ab}	15.39±1.51^{b}	34.03±4.1^{ab}	75.01±0.37^{b}	8.23±0.42^{a}	9.51±0.55^{b}	77.29±4.55^{b}	115.68±7.8^{b}	1.7٤٦±0.09^{c}	1.74٩±0.09^{c}
Col.(0.14mg) + benzene 5th	9.18±0.63^{bc}	9.70±1.41^{c}	29.97±2.9^{bc}	59.07±4.63^{c}	6.40±0.35^{b}	8.06±0.47^{c}	65.65±3.88^{c}	97.99±6.62^{c}	2.3٥٩±0.12^{b}	2.3٦٥±0.12^{b}
Col.(0.21mg) + benzene 6th	8.29±0.57^{c}	11.77±1.15^{c}	24.75±4.1^{c}	53.33±4.18^{c}	5.78±0.32^{b}	7.28±0.42^{d}	59.27±3.50^{c}	88.46±5.98^{d}	2.7٨٩±0.13^{a}	2.7٩٣±0.13^{a}

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

4. DISCUSSION

The obtained data reveled that rats which administrated benzene caused significant increase in WBCs count (leukemia) and decreases in Hb level and platelets number compared with control groups . These results come in accordance with (El Harthy 2010) who reported that Chronic exposure of human to benzene is associated with disorders including a plastic anemia which result from bone marrow toxicity of benzene causing progressive decrease in erythrocyte ,thrompocytes and each of the various type of leukocyte. Moreover (Beggs et al., 2012) found that Leukopenia associated with long-term colchicine administration in accordance to present study as WBCs count decreases after treatment with colchicine as the lowest value with the highest concentration of colchicine (0.21mg/kg/wt).

The obtained data showed that rats which administrated benzene expressed decrease in CAT, GSH, SOD, GPx and increases in MDA level compared with control groups similar to (Dewi and Yuniastuti 2016) who found that the longer employees work in fuel stations, the more exposure and accumulation of benzene, toluene, and xylene compound so that it tends to decrease antioxidant in the body as SOD content of fuel stations operators was lower than SOD content of control. In addition, (Odewabi et al., 2014) research at Nigeria stated that free radical exposure on FFS employees increased MDA content and reduced antioxidant content in blood significantly than control. And after treatment with colchicine in our study we found that CAT, GSH, SOD,

GPX and MDA level were decreased after 2and 4 weeks compared with control and benzene group in accordance to (Ganguly et al., 2005) who studied that colchicine induced experimental Alzheimer model in rats, decreased the SOD and CAT activity significantly.

The obtained data showed that rats which administrated benzene had increases in IL-2, IL-6, AFP and TNFα ,such finding was in consistence with (Hong et al.,2007) who showed that high levels of circulating IL-6 are observed in almost every type of tumor. Also (Zhou et al.,2017) who indicated that higher level of TNF-α expression tends to be associated with adverse clinical features and refractory disease in leukemia, moreover (Burhan 2016) found that there was increased significant of AFP in patients with leukemia.

In our present study after injection of colchicine with different concentrations, levels of IL-2, IL-6, AFP and TNFα decreases after 2and 4 weeks similar to (Martínez et al., 2015) who demonstrated a marked reduction in local IL-6 production as well as venous levels with colchicine and (Leung et al., 2015) who revealed significant reduction in interleukin and TNFα.

Mean of Caspase-3 decrease after administration of benzene and this similar to (Sun et al., 2014) who stated that benzene metabolites induced dysregulation of apoptosis due to caspase-3 inhibition, which contributes to carcinogenesis , where after treatment with colchicine with different concentrations mean of caspase-3 significantly increase after 2 and 4 weeks compared with benzene group as the highest value of caspase-3 with the highest concentration of colchicine (0.21mg/kg/wt.) and this results come in accordance with (Chen et al., 2012) who stated that colchicine's effect on cell apoptosis was mediated by the activation of caspase-3.

5. CONCLUSION

Colchicine is well-tolerated medicine agent, and finds wide application worldwide.We have a tendency to counsel to the medical practitioner community the leukemogenic effect of colchicine. Overall, we tend to found that more analysis for

advantages and harms of colchicine. Our findings ought to thus be taken with caution.

6. REFERENCES

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