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HAVE *GLYCYRRHIZA GLABRA* & COLCHICINE CYTOTOXIC EFFECT ON PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA

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ABSTRACT

Background: Chronic myelogenous leukemia is a malignant clonal, myeloproliferative disorder of the pluripotent hematopoietic stem cells. the objective to evaluate *in vitro* the cytotoxic effect of both *glycyrrhiza globra* (licorice) and colchicne on normal myeloid stem cell & leukemic myeloid cells of patients with chronic myelogenous leukemia. The serial dilution of aqueous licorice extraction & colchine in the concentration (250,125,68.5,31.25,15.625,7.8,25 µg/ml) were added to the cell culture. Cells viability was assessed by MTT assay. **Results:** The obtained results shamed that the toxic effect (inhibitory rate of growth) of both licorice & colchicines urea in dose e and time dependent. There were significant difference (P<0.05) between concentration & time exposure (24, 48,72hr). the highest inhibition rate of licorice & colchicines on the leukemic cells were 75.50% & 75.16% respectively in concentration 250 µg/ml & the loment inhibition rate were 40.49% & 43.74% respecters in concentration 7.8125 µg/ml for exposure time 72 hr while the highest inhibition rate of licorice & colchicines on the normal myeloid cell were 41.82% 837.22% respectively in concentration 250µg/ml & the lowest inhibition rate were 13.17% & 17.21% respectively in concentration 7.8125 µg/ml for exposure time 72hr. conclusion both licorice & colchicines have potent growth inhibition for leukemia myeloid cells & less inhibition for normal cells.

KEYWORDS: Glycyrrhiza globra-licorice, colchicine, normal & leukemic myeloid cells culture.

INTRODUCTION

Chronic myelogenous leukemia (CML) is a type of leukemia that occurs in about 20% of all types of leukemia.[1] It is a malignant clonal myeloproliferative disorders of pluripotent hematopoietic stem cells[2], characterized by formation of specific abnormal chromosome such us Philadelphia chromosome in 95% of patients.[3] This malignant disease occurs mostly in adult patients & rarely in children.[4]

A recent study proved that herb of camellia sinensis (green tea) has cytotoxic effect on the leukemic and normal myeloid stem cells of patients with CML.[5]

The present study performed to explore the possible cytotoxic effect of aqueous extract of licorice & colchicines which is the extract of herb-on normal & leukemic myeloid cells of patients with CML.

Glycyrrhiza globra. L (Licorice) is related to plant family of Papi lionaceae, it is one of the most biologically active herbs. Its constituents include saponins like glycyrrhizic acid, flavonoids like chalcones & polyphenols the glabridin related to isoflavones stilbenoids fatty acid & glucose.[6,7] All these compounds are present in the unpeeled dried roots & the rhizomes of plant which is cultivated in china, India, Iraq, Turkey, Greece, Egypt & other .Licorice had been used in gastric ulcer.[8] It has hepatoprotective effect against toxicity induced by CCL_4 in rabbits[9] Has anti-inflammatory effect similar to hydrocortisone used in management of bronchial asthma.[10] It has anticancer activity & may increase the sensitivity of tumors to anticancer drugs.[11]

Colchicines is an alkaloid extracted from herb of colchicum autumnale (auntumn crocus) used to decrease the frequency of cute got attack & relieve pain with beneficial effect in acute Mediterranean fever, Behcet syndrome & liver cirrhosis.[12] It inhibits cell division by binding to the intra cellular protein tubulin.[13]

MATERIALS AND METHODS

Chemicals

Colchicine was used in present study after dissolve in water supplied by (Galeniqum vermin lab. France) batch No. 2C56R.

Plant extraction

Licorice was purchased from well-known herbal bureau (AL-Medina) in Baghdad city & was identified and authenticated by Iraqi national institute for herbs. The unpeeled dried roots & the runners were cleaned carefully & powdered by an electrical grinder then passed through sieve no.4 to remove the debris. The sieved powder was stored in airtight black container at room temperature. The aqueous extract was prepared by diluting one volume of well grinded powder to ten volume of water at 80^{o}C in a stoppered flask after shaking completely. Then, it was allowed to stand for 10 minutes to be cold & filtered for practical purpose. The aqueous extract should be used with 12 hours.[14]

Subjects

Fifteen patients with CML and 15 healthy individuals with normal myeloid cells. Both of normal & leukemic were 8 males and 7 females & their ages ranged between 42-68 years who attended AL-Kadhimain teaching hospital & National center of hematology in the period from September 2014 to the September 2015 and this study was performed in the Iraqi center of cancer & medical genetics research.

Human bone marrow obtained from the posterior iliac crest by using aspiration needle under local anesthesia (10 ml lidocaine). The specimen was taken in EDTA anticoagulant tube for culture.[15] Ficoll-opaque was added slowly for the isolation of myeloid cell.[16]

Later, the cells were placed in to 25 cm falcon after adding 10ml of RPMI-1640 (20%FCS). This medium was prepared by dissolving 16.35g powder of RPMI-1640 with HEPES and L-glutamine. Added 2g of sodium bicarbonate powder, 1ml of ampicillin, 0.5 ml of streptomycin & 200ml of fetal calf serum (20%FCS) were added to one liter of medium in cub a ted at 37^{o}C.[17] The same procedure was used for normal & leukemic myeloid cells culture & the viable cells count was 52×103 cells.

Licorice & colchicine were prepared in six concentration (250, 125, 62.5, 31.25, 15.625, and 7.8125 µg/ml) by using the maintenance medium (RPMI-1640 serum free medium) to complete the volume. After the growth of myeloid cells (normal & leukemic cells) in falcone at 37^{o}C & reaching to confluent mono layer that as examined under the inverted microscope. Then 200µl of licorice and cholchicine with concentration (250,125, 62.5, 31.25, 15.625, and 7.8125µg/ml) were added to the cells culture (200µl of cell suspension in the each well of micro titration plate of 96 wells flat bottom). Four replications were used for each

concentration of drug, incubated at37C̊ for selected time (24, 48, 72 hr.) for the leukemic cells & 72hr. for normal cells.[18]

Methyl thiazolyl tetrzolium (MTT) the detection of the cells viability was assessed by MTT assay which is based on the reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) by mitochondrial dehydrogenase enzyme of intact cells. MTT solution of 28µl (2mg/ml) was added to calculate cells viability. Read at 550 nm by ELLSA reader.[19] The percentage of inhibition rate for cells growth was calculated as: A-B/Ax100. A: is the mean of optical density for untreated well (control). B: is the mean of optical for treated wells.[20]

Statistical analysis: the descriptive data of the results was demonstrated as means, percentages, ranges, standard errors & LSDS (P≤0.05) for comparison.[21]

RESULTS

The present study showed that the inhibition of growth rate (cytotoxic effect) of both Licorice & colchicines on the leukemic myeloid stem cells was dose and time dependent.

Table 1: Inhibition rate of licorice & colchicine on the leukemic myeloid cells culture.

Conc. (µg/ml)	Licorice Mean ± SEM			Colchicines Mean ± SEM			LSD value
	24 hr.	48 hr.	72 hr.	24 hr.	48 hr.	72 hr.	
7.8125	25.69 ± 2.08	30.61 ± 0.42	40.49 ± 2.33	18.60 ± 0.63	29.91 ± 1.45	43.74 ± 0.46	4.266*
15.625	33.44 ± 1.09	37.00 ± 1.33	48.66 ± 1.21	22.63 ± 0.88	33.33 ± 0.49	53.80 ± 1.77	5.072 *
31.25	39.91 ± 1.28	42.85 ± 0.98	58.60 ± 1.73	27.97 ± 0.38	38.28 ± 0.45	62.68 ± 1.34	4.763 *
62.50	45.19 ± 0.80	46.41 ± 0.34	64.32 ± 0.78	32.96 ± 0.86	41.85 ± 0.99	67.56 ± 0.63	4.984 *
125	49.23 ± 1.04	51.41 ± 0.56	70.60 ± 0.76	38.40 ± 1.33	45.90 ± 0.78	74.11 ± 0.52	4.247 *
250	52.85 ± 0.79	57.17 ± 1.32	75.50 ± 1.99	46.14 ± 0.77	54.86 ± 0.91	75.16 ± 0.21	6.033 *
* (P≤0.05).							

* = significant differences.

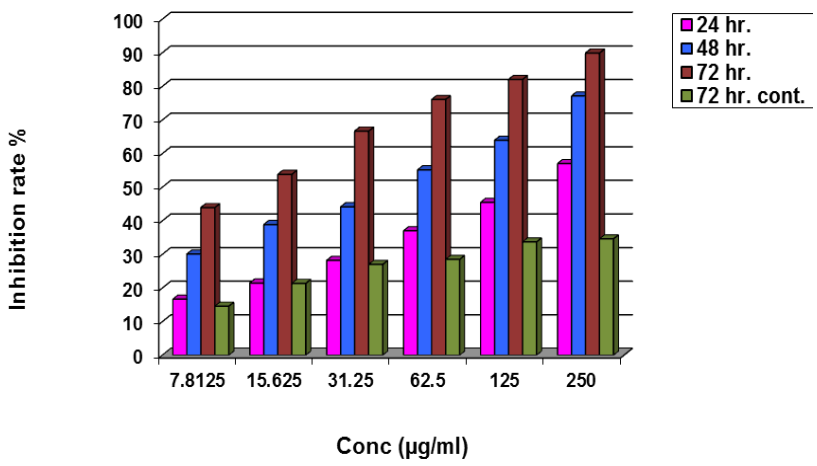

Figure 3.37: Inhibition rate of imatinib on the leukemic myeloid cells in human at 24,48,72hrs of exposure and normal at 72hrs.

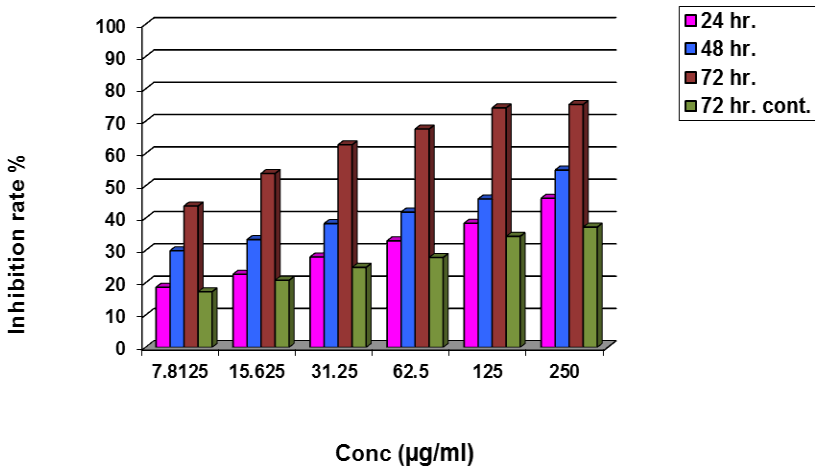

Figure 3.38: Inhibition rate of colchicine on the leukemic myeloid cells in human at 24, 48,72hrs of exposure and normal at 72hrs.

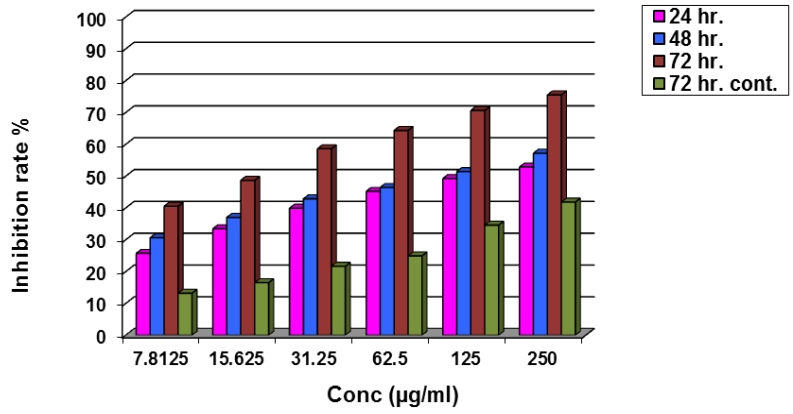

Figure 3.41: Inhibition rate of licorice on the leukemic myeloid cells in human at 24, 48,72hrs of exposure and normal at 72hrs.

There were significant differences (P≤0.05)between concentration & time (24,48,72hr) as seen in table-1 while on the normal myeloid cells depending on the concentration, thus increased the effect with the increased concentration at 72hr. and the inhibition rate of licorice & colchicines were 13.172%, 17.942% 21.121%, 24.863%, 33.652%, 41.232% respectively to licorice versus 17.218%, 20.324%, 24.621%, 28.786%, 33.251%, 37.235% respectively to colchicines for (7.8125, 15.625, 31.25,62.5,125,250 µg/ml) in comparison to inhibition rate on leukemic cells. See figure 1 and 2.

DISCUSSION

The present study demonstrated highly effect of both licorice & colchicines on the leukemic cells & less effect on the normal myeloid cells. The classical method evaluating the effect of herb or drug on the cells is based on proportion of inhibition that indicates the rate of inhibition of the cell growth or percentage of toxicity.

The disorder of the pluripotent hematopoietic stem cell is characterized by the t (9:22) Philadelphia chromosomal translocation leading to the production of BCR-ABL fusion. This important cause of CML & it present in up to 95 to 95% of patients with CML[22] that is inhibited by lmatinib. This drug can inhibit tyrosine Kinase domain of BCR-ABL oncoprotein & prevents phosphorylation of the Kinase substrate.[23]

The good effect of licorice on bone marrow is related to the presence of important major compounds such as flavonoids, terpenoids, saponins & isoflavones.[11] The anticancer effect of licorice is mediated by a flavonoid-licochalcone with apoptotic effect & has ability to decrease the level of bcl-2 & increase the sensitivity of tumors to anticancer drugs. It inhibits proliferation of T cells & production of cytokines[24], also the polyphenolic compounds of flavonoid have important effect on cancer chemotherapy by different mechanisms such as inactivation of carcinogen, induction of apoptosis differentiation, anti-angiogenesis, anti-proliferation and arrest of cells cycle.[25] Also Moosavi and coworkers reported that carbenoxolone- a derivative of licorice- induces apoptosis and inhibits survivin & survivin-Δ EX3 genes expression in human leukemia K562 cells.[26]

The inhibitory effect of colchicine on growth of tumor cells is related to its inflammatory effect by binding to intracellular protein tubulin, thereby its polymerization into micro tubules & leading to the inhibition of leukocytes migration & phagocytosis also inhibits macrophages release of inflammatory mediators PG, inter leukemia -1 & leukotriene B.[13]

Colchicines inhibits mitosis or cell division through disrupting the micro tubules that pull the cell apart during division.[27] Cancer cells are more sensitive to inhibition of mitosis than normal.[28]

The effect of licorice & colchicines was simile to the effect of imatinib on the leukemic & normal myeloid cells culture but by different mechanisms.[29]

CONCLUSION

Licorice & colchicines both have a highly inhibited growth for the leukemic myeloid stem cells & less inhibitor for the normal cells. This is a good indicator for selecting any drug with high cytotoxic effect on the leukemic cells & less cytotoxic effect on the normal cells.

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