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ANTICANCER ACTIVITY OF LAMOTRIGINE AND 5-FLUOROURACIL INDIVIDUALLY AND IN COMBINATION USING CHRONIC MYELOID LEUKEMIA CELLS I.E. K562 CELLS AND COLON CANCER CELLS I.E. COLO320 CELLS

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ABSTRACT
The anticancer activities of different concentrations of Lamotrigine individually and in combination with 5-Fluorouracil were investigated for in vitro cytotoxicity by trypan blue exclusion method using k562 and Colo 320 cell lines. Lamotrigine individually had shown the potent anticancer activity and significantly on k562 cell lines at 100µM, but efficacy is more in combination of Lamotrigine and 5-Fluorouracil and lesser than 5-Fluorouracil on both the cell lines. In additional to this a safe MTD of combination of Lamotrigine and 5-Fluorouracil was also determined in Swiss albino mice for further in vivo anticancer activity. The toxicity of the both the drugs has been reduced remarkably at this concentration.
Key words: Anticancer activities, Lamotrigine, 5-Fluorouracil, k562, Colo 320 cell lines

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INTRODUCTION

Cancer is a disease characterized by uncontrolled growth and spread of body's own cells. It is one of the major causes of death in developed countries. One in three people will be diagnosed with cancer during their life time. Neoplasm simply means 'new growth' and neoplasms may be benign or malignant (1). Non-malignant or benign tumors are much more common than malignant tumors. A benign tumor is a limited growth of cells that seems to be under some sort of control. In a malignant tumor the cells look less like the cells from which they developed. The term anaplasia is used to describe cells that have lost their distinctive features. The multiplication of cells also

continues without control. In the body, superfluous or unwanted cells are destroyed by a pathway called apoptosis, or programmed cell death. In this suicidal process, the cell shrinks, the chromatin condenses, and the nucleus fragments. The cell membrane forms blebs (out pouches), and the cell breaks up into membrane-enclosed apoptotic vesicles (apoptotic bodies) containing varying amounts of cytoplasm, organelles, and DNA fragments. Phosphatidylserine, a lipid on the inner leaflet of the cell membrane, is exposed on the external surface of these apoptotic vesicles is one of the phagocytic markers recognized by macrophages and other nearby phagocytic cells that engulf the apoptotic bodies. Apoptosis is a normal part of multiple processes in complex organisms: embryogenesis, the maintenance of proper cell number in tissues, the removal of infected or otherwise injured cells, the maintenance of the immune system, and aging. Apoptosis can protect organisms from the negative effect of mutations by destroying cells with irreparably damaged DNA before they proliferate. The failure of apoptosis to remove excess or damaged cells can contribute to the development of cancer (2).

Colon cancer is a neoplastic disease of the large intestine, which can be derived from both inherited and somatic genetic alterations that develop over the course of a lifetime. Colon cancer is a disease of the large intestine which begins at a structure called the caecum, located in the right lower quadrant of the abdomen, and continues through all portions of the abdomen to its junction with the rectum, located in the deep pelvis. Prevention together with early detection and treatment are the keys to a successful outcome for this very common disease (3).

Chronic granulocytic leukemia or chronic myelogenous leukemia it is a type of cancer that starts in the blood-forming cells of the bone marrow and invades the blood. There are three phases of CML: chronic, accelerated, and blastic. The chronic phase is least aggressive, and characterized by no or mild symptoms; the accelerated phase has noticeable symptoms, such as fever, poor appetite, and fatigue; the blastic phase is most aggressive with more severe symptoms that may also include an enlarged spleen. CML is most common in adults, but can also occur in children.

In the past 10 years, the standard of care for CML is to treat with a type of targeted drug called a tyrosine kinase inhibitor (such as Imatinib [Gleevec]). There are several new targeted tyrosine kinase inhibitors under development and targeted to the underlying causes of Imatinib resistance and disease progression like Dastinib, Nilotinib, SKI-606, VX-680, BIRB-796, ONO 12380. Most of these new molecules have shown promising in vitro activity against a subset of BCR-ABL mutants and also might suppress the proliferation of Imatinib-resistant cells in which the cause of the resistance is over expression of BCR-ABL (4, 5).

The aim and objective of the present study was to evaluate *in vitro* anticancer activity of Lamotrigine and 5-Fluorouracil individually and in combination using Chronic Myeloid Leukemia cells i.e. K562 cells and Colon cancer cells i.e. COLO320 cells.

MATERIALS AND METHODS

Cell lines used

K562 cells (Chronic Myeloid Leukemia) and COLO 320 cells (Colon cancer) were obtained from Sugen life sciences Pvt. Ltd. Tirupati A.P.

Drugs used

Lamotrigine was obtained from RACHEM Pvt. Ltd Hyderabad, A.P, Fluorouracil injection 500 mg/10 ml was collected from Vishwa Bharati multi specialty hospitals Kurnool, A.P.

METHODS Experimental Design

Table-1 Experimental design for MTD determination

GROUP	DOSE mg/kg		DOSE VOLUME	NO.OF ANIMALS
	LAMOTRIGINE	5-FLUROURACIL		
Control	-	-	10ml/kg	3
GROUP1	269	115		3
GROUP 2	201.75	86.25		3
GROUP 3	134.5	57.5		3
GROUP 4	67.25	28.75		3
GROUP 5	134.5	28.75		3
GROUP 6	67.25	57.5		3

Procedure

Female healthy and diseased free Swiss albino mice of weight 20-25 gm were selected and kept for a quarantine period for a week. After quarantine period the animals were grouped in to three animals per group and kept fasted for about 3-4 hours prior to dosing and 1 hour after dosing. Since the adequate data was available for the both the drugs the maximum tolerated dose was determined by starting dose at their LD50 values.

LD50 of Lamotrigine - Oral LD_{50} (Mouse) = 269 mg/kg (6).

LD50 of 5-Fluorouracil - Mouse Oral LD_{50} 115 mg/kg (7).

The mice were treated with lamotrigine and 5-fluorouracil at their respective LD50 values. If the animals show any mortality or clinical symptoms the doses were reduced to 25% from their prior dosing values and administered to another group of mice. The dose reduction was stopped when the animal shows no signs of toxicity.

A single cross over study performed between the safe dose and its prior toxic dose. The Mice were observed individually after dosing for signs of toxicity and mortality at least once during the first 30 minutes, 1, 2, 3 and 4 hours. Subsequently, the Mice were observed twice a day for morbidity and mortality for a period of 14 days. Individual body weight was recorded for all animals on the day of commencement of treatment, weekly intervals and on the day of sacrifice.

After observation period the blood was collected from the mice by retro orbital sinus puncture for hematological and biochemical parameters.

Haematological and Biochemical Analysis

Hematological analysis was performed using blood samples from mice. Hematological parameters like hemoglobin concentration, hematocrit, erythrocyte count, total leukocyte, platelet count, mean corpuscular hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration was analyzed. Biochemical parameters like SGOT, SGPT were analyzed.

RESULTS AND DISCUSSION

In the present study anticancer activity of lamotrigine have been determined by treating colo320 cells and K562 cell lines. Since the *invitro* cell lines are the significant source for screening of many drugs for its efficacy and potency. The cell lines are treated with Lamotrigine at different concentrations it had shown good cell growth inhibition effect at higher concentration and in combination with 5-fluorouracil. Relatively lamotrigine had shown better cell growth inhibition on K562 cell lines than on COLO320 cell lines. And also a safe and maximum tolerated dose of combination of lamotrigine and 5-fluorouracil in swiss albino mices was also determined by acute toxicity studies.

% of viability values of lamotrigine and 5-flurouracil on K562 cells:

% of viability values of lamotrigine and 5-flurouracil on K562 cells was shown in table-2.

Table-2 % of viability values of Lamotrigine and 5Flurouracil on K562 cells.

S.No	Lamotrigine(L) in µM	Combination in µM (L + 5FU)		5-Fluorouracil (5FU) in µM	% of viability				
		L	5FU		Control	Negative Control	L	L+5FU	5FU
1					100	95.63			
2	10	5	5	10			74.04±3.66 *	67.27±3.19 *	30.63±1.86*

							74.04±2.11 **	67.27±1.84 **	30.63±1.07**
3	25	12.5	12.5	25			66.71±1.36 * 66.71±0.78 **	55.41±1.92 * 55.41±1.10 **	20.13±2.02* 20.13±1.16**
4	50	25	25	50			63.61±2.24 * 63.61±1.29 **	48.46±1.78 * 48.46±1.02 **	1.3±0.31* 1.3±0.18**
5	100	50	50	100			58.96±2.07 * 58.96±1.19 **	37.21±1.80 * 37.21±1.04 **	0.18±0.31* 0.18±0.18**

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		L	5FU		Control	Negative Control	L	L+5FU	5FU
1					100	95.63			
2	10	5	5	10			74.04±3.66* 74.04±2.11**	67.27±3.19* 67.27±1.84**	30.63±1.86* 30.63±1.07**
3	25	12.5	12.5	25			66.71±1.36* 66.71±0.78**	55.41±1.92* 55.41±1.10**	20.13±2.02* 20.13±1.16**
4	50	25	25	50			63.61±2.24* 63.61±1.29**	48.46±1.78* 48.46±1.02**	1.3±0.31* 1.3±0.18**
5	100	50	50	100			58.96±2.07* 58.96±1.19**	37.21±1.80* 37.21±1.04**	0.18±0.31* 0.18±0.18**

MEAN±S.D*; MEAN±S.E **.

% of viability values of Lamotrigine and 5 Flurouracil on colo320 cells

% of viability values of Lamotrigine and 5 Flurouracil on colo320 cells is shown in table-3.

Table-3 % of viability values of lamotrigine and 5-Flurouracil on colo320 cells

S.No	Lamot-rigine(L) in µM	Combination in µM (L + 5FU)		5-Fluoro-uracil (5FU) in µM	% of viability				
		L	5FU		Control	Negative Control (DMSO)	L	L+5FU	5FU
1					100	97.77			
2	10	5	5	10			82.96±1.28*	73.52±4.45*	52.4±1.64*

							82.96±0.74* *	73.52±2.57** *	52.4±0.95
3	25	12.5	12.5	25			74.81±1.12 74.81±0.65	59.25±1.28 59.25±0.74	23.86±0.99 23.86±0.57
4	50	25	25	50			67.4±2.56 67.4±1.48	50.36±1.74 50.36±1.00	4.71±3.12 4.71±1.80
5	100	50	50	100			64.95±0.44 64.95±0.25	48.14±1.28 48.14±0.74	0±0 0±0

MEAN±S.D*; MEAN±S.E **.**

Determination of maximum tolerated dose

Physical signs of toxicity

The group-1 mice which received Lmotrigine(269 mg/kg) and 5-fluorouracil(115 mg/kg) had shown the signs of toxicity like Somatomotor incordination, Tachypnea, Tachycardia, loss of Rightening reflex the mouse had reached the moribund condition for within 16-18 hours the animal was humanely sacrificed and was observed for the weight variation, hematological and biochemical observations .

Due to the above signs of toxicity the group-2 mice were administered with Lmotrigine(201.75 mg/kg) and 5-fluorouracil(86.25 mg/kg) orally and the mouse had shown the signs of toxicity like Somatomotor incordination, Tachypnea, Tachycardia, loss of Rightening reflex the mouse had reached the moribund condition for within 24-26 hours the animal was humanely sacrificed and was observed for the weight variation, hematological and biochemical observations.

Due to the above signs of toxicity the group-3 mice were administered with Lmotrigine(134.5 mg/kg) and 5-fluorouracil(57.5 mg/kg) orally and the mouse had shown the signs of toxicity like Somatomotor incordination, Tachypnea, Tachycardia, loss of Rightening reflex but, the mouse had shown the signs of recovery after 6 hours of drugs administration and then completely recovered and the animal was observed for 14 days for any signs of toxicity and weight variation and after the 14 days time period of observation the mouse were observed for the weight variation, hematological and biochemical observations.

Due to the above signs of toxicity the group-4 mice were administered with Lmotrigine (67.25 mg/kg) and 5-fluorouracil (28.75 mg/kg) orally and the mice had shown the signs like Somnolence, Lethargic the animal was observed for 14 days for any signs of toxicity and weight variation and after the 14 days time period of observation the mice were observed for the weight variation, hematological and biochemical observations. Now a single cross over study was done between the last safe and its prior toxic dose.

Group-5 mice were treated with Lmotrigine(134.5 mg/kg) and 5-fluorouracil(28.75 mg/kg) the mice had shown the signs of toxicity like Somatomotor incordination, Tachypnea, Tachycardia, loss of Rightening reflex, circling movements the mouse had shown the signs of recovery with in a time period of 10-12 hours and the mouse had got recovered from the toxic signs. The mice were kept in observation for a time period of 14 days. After the completion of 14 days of time period the mice were observed for the weight variation, hematological and biochemical observations.

The mouse which received Lmotrigine (67.25 mg/kg) and 5-fluorouracil (57.5 mg/kg) had shown no signs of toxicity and the animal was kept for observation for about 14 days for signs of toxicity and weight variation. After the 14 days time period of observation the mice were observed for the weight variation, hematological and biochemical observations.

Table-4, 5 and 6 shows hematological, biochemical and weight variation observations.

Table-4 Hematological results of various dose treated groups of mices

Group	RBC (10^6/µL)	WBC (10^3/µL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (Pg)	MCHC (g)	PLT (/µL)
Normal value	5.50-6×10^6/µL	3.0-14.2×10^3/µL	10.9-16.3 g/dL	38.5-45.1%	48-56 fL	20-25Pg	30-38g	1084-1992/µL
control	5.77±0.04* 5.77±0.02**	13.41±0.56* 13.41±0.32**	16.1±0.36* 16.1±0.20**	43.74±0.85* 43.74±0.49**	53.8±1.75* 53.8±1.01**	23.19±0.67* 23.19±0.38**	35.03±0.30* 35.03±0.17**	25±2* 25±1.15**
Group1	4.71±0.23* 4.71±0.13**	12.23±0.35* 12.23±0.20**	13.39±0.41* 13.39±0.24**	36.26±0.43* 36.26±0.24**	49.41±0.31* 49.41±0.18**	22.41±0.58* 22.41±0.33**	31.08±0.26* 31.08±0.15**	27.67±0.57* 27.67±0.33**
Group2	5.18±0.15* 5.18±0.08**	12.96±0.12* 12.96±0.07**	14.87±0.36* 14.87±0.20**	41.34±0.34* 41.34±0.19**	53.13±032* 53.13±0.18**	21.97±0.17* 21.97±0.10**	32.36±0.33* 32.36±0.19**	28.33±0.57* 28.33±0.33**
Group3	5.48±0.34* 5.48±0.2**	13.35±0.38* 13.35±0.21**	14.74±0.11* 14.74±0.66**	41.92±0.19* 41.92±0.11**	48.4±0.83* 48.4±0.48**	22.81±0.36* 22.81±0.21**	34.31±0.40* 34.31±0.23**	18.67±0.57* 18.67±0.33**
Group4	5.64±0.12* 5.64±0.06**	12.4±0.34* 12.4±0.19**	15.49±0.32* 15.49±0.18**	43.25±0.81* 43.25±0.46**	53.44±0.35* 53.44±0.20**	23.32±0.28* 23.32±0.16**	35.49±0.30* 35.49±0.17**	21±0* 21±0**
Group5	5.59±0.15* 5.59±0.09**	13.54±0.07* 13.54±0.04**	15.32±0.38* 15.32±0.04**	42.69±0.38* 42.69±0.21**	52.27±0.57* 52.27±0.33**	22.43±0.43* 22.43±0.25**	35.04±0.24* 35.04±0.13**	18.33±0.57* 18.33±0.33**
Group6	5.68±0.23* 5.68±0.13**	13.25±0.15* 13.25±0.08**	15.49±0.49* 15.49±0.28**	42.63±0.49* 42.63±0.28**	51.08±0.37* 51.08±0.21**	22.13±0.33* 22.13±0.19**	35.21±0.33* 35.21±0.19**	23.67±0.57* 23.67±0.33**

Table-5 Biochemical variation results of various dose treated groups of mices

S.No	SGOT(U/L)	SGPT(U/L)
Control	34±0.25* 34±0.14**	21.67±0.66* 21.67±0.38**
Group1	38.3±0.13* 38.3±0.07**	26.1±0.36* 26.1±0.21**
Group2	37.32±0.35* 37.32±0.20**	25.11±0.23* 25.11±0.13**
Group3	33.26±0.55* 33.26±0.32**	22.09±0.27* 22.09±0.15**
Group4	32.26±1.08* 32.26±0.62**	19.11±0.20* 19.11±0.11**
Group5	33.16±0.11* 33.16±0.06**	21.76±0.86* 21.76±0.49**
Group6	31.75±0.35* 31.75±0.20**	18.45±0.41* 18.45±0.23**

Table-6 Weight variation results of various dose treated groups of mices

S.No	Weight in gm		
	0 day	7 days	14days
Control	24.8 25.12 22.7	26.03 27.13 23.37	27.5 29.08 25.01
Group1	24.72 23.08 20.5	- - -	- - -
Group2	22.69 19.93 22.37	- - -	- - -
Group3	24.42 23.84 23	25.73 25.04 22.6	27.04 26.36 23.62
Group4	21.41 22.09 23.80	22.33 23.21 24.40	24.69 24.41 25.73
Group5	24.62 24.01 23.2	25.92 25.71 24.8	26.64 27.57 24.19
Group6	23.67 24.12 24.30	24.92 25.94 25	25.25 27.56 26.07

CONCLUSION

In the present investigation the anticancer activity of Lamotrigine, 5-Fluorouracil and in combination of both the drugs was determined on Colo 320 and K562 cell lines by Trypan blue exclusion method. It was observed and determined that Lamotrigine individually had exhibited the anticancer effect on both the cell lines at all different concentrations but, significantly more efficacious at higher concentrations (100µM). Relatively the antineoplastic activity of Lamotrigine individually was more efficacious on K562 cell line rather than on Colo320 cell line. But, the combination effect of both the drugs is relatively higher than Lamotrigine alone on both the cell lines and more efficacious on K562 cell lines and lower, than 5-Fluorouracil on both the cell lines. And also the maximum tolerated dose (MTD) of combination of Lamotrigine and 5-Fluorouracil was determined; various dose ranges had exhibited various toxicities. The safe doses or MTD of combination was determined, and are Lamotrigine (67.25 mg/kg) and 5-Fluorouracil (57.5). The toxicity of the both the drugs at this dose range of MTD was highly minimized.

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