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Evaluation of Anti-Leukemic Activity of Allium Sativum and Vitis Vinifera for Synergistic Action on Benzene-Induced Acute Leukemia on Male Sprague-Dawley (SD) Rats

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Abstract **Original Research Article**

Acute leukemia is a cancer of blood cells that characteristically comes suddenly and if not treated, progresses quickly. In acute leukemia, the leukemic cells do not mature properly at a normal time but rapid development of blast cells was observed. The aim of the present study is evaluation of anti-leukemic activity of *Allium sativum* and *Vitis vinifera* for synergistic action on benzene induced acute leukemia on sprague-dawley (SD) rats. The commercial garlic bulbs (*Allium sativum*) and grape fruits (*Vitis vinifera*) are collected, Authenticated and extracted separately with n-hexane and distilled water. The both extracts of garlic and grapes were studied for acute oral toxicity as per revised organization for economic cooperation and development guide lines number 423. Leukemia was successfully induced in SD rats by intravenous injection of benzene. The base line and post analytical blood samples was collected and analyzed for hematological parameters.The outcomes of hematological parameters in various experimental groups of murine model demonstrated anti leukemic effect of both plant extracts. The combination of these two plant extracts shows better efficacy then individual extracts but not more than or equal to standard drug that is 5- fluorouracil (15 mg/kg).The both extracts of garlic and grapes are ability to promote the phagocytosis of leukemic blast cells and then reduce the acute leukemia growth. It demonstrated anti leukemic potential that might be due to the presence of alkaloids, glycosides, tannins, Poly phenolics, and flavonoids in both plant extracts.

Keywords: Acute Leukemia, Synergistic Action, Benzene, Anti Leukemic Action, Toxicity, Phagocytosis.

INTRODUCTION

Acute leukemia is characterized by a rapid increasing the number of immature white blood cells is called as blast cells.. Its results bone marrow unable to produce healthy white blood cells. Acute leukemia is a common type of leukemia in children (NB *et al*). Acute leukemia was mainly classified into two types acute myloid leukemia (AML) and acute lymphoid leukemia (ALL).the most common symptoms in children are easy bruising, pale skin, fever, enlarged spleen and liver [1-3].The known causes of this disease is mainly radiation, viruses like human t-lymphotropic virus, chemicals and drugs like benzene and chemotherapy agents. Diagnosis of acute leukemia is mainly repeated complete blood counts and then take it out samples from bone marrow for leukemic blast cell count and find out the stage and severity of a disease [4-6].

Most forms of leukaemia are treated with a multi-drug chemotherapy regimen. Some are also treated with radiation therapy and some are treated with radiation and bone marrow transplantation. Phytochemicals are obtained from plant sources, its having anti cancer and anti oxidant properties to fight against to the cancers and protect our body. Plants like *Allium sativum* (garlic), and *Vitis venifera* (grapes) contains phytochemicals like alkaloids, glycosides, flavonoids, and Polyphenolics etc. [7,8]. Flavonoids are abundantly present in strawberries, grapes, apples, onions etc are rich in anti-cancer properties.

It causes cancer cell death by increasing nitric oxide levels leading to breaks in DNA and activation of apoptotic pathways in acute monocytic leukemia. Phytochemicals not only increases the efficacy of therapy, but it has been reported that a combination of various phytochemicals may be a better option in

imparting cytotoxicity to cancer cells (BabiorBM Oxygen-dependent microbial killing by phagocytes) [9-12].

MATERIALS AND METHODS

Allium sativum extraction procedure

Collection of sample and sample treatment

The garlic bulbs were obtained from guntur local market, guntur, Andhra Pradesh state, India and identified by a taxonomist at botany department Acharyanagarjuna university of science and technology. The sun dried garlic was pounded by using motor and pestle. The powdered garlic was sieved and stored in a covered plastic container.

Oil extraction

The extraction of garlic oil was conducted with a soxhlet extractor using n-hexane (boiling point of 40^0c -60^0c) for six hours. After six hours of continuous extraction, solvent was removed by means of distillation. Garlic extract was collected and stored at 2^0 C for phytochemical analysis [13,14]. Percentage yield was determined by using following formula (Harborne JB. Phytochemical Method: A Guide to Modern Techniques of PlantsAnalysis. New York: Chapman and Hall).

$$\text{Oil content (\%)} = \frac{\text{volume of the oil}}{\text{Weight of sample}} \times 100\%$$

Vitisvenifera extraction procedures

Plant material

The sample of grapes was purchased at a guntur local market, guntur, Andhra pradesh, India. In late october 2018 and identified by a taxonomist at botany department acharyanagarjuna university of science and technology.

Extraction procedure

The fresh grapes were mashed, and collected the 10 gm of crushed grapes.it was mixed with 20 ml of the distilled water (extraction solvent) 0.1 ml / 10 ml of solvent (v/v) of concentrated Hcl to avoid oxidation of the phenolic compounds. the complex mixture is placed in a water bath with continous stirring [15,16]. After its allows for centrifugation at 3000 rpm/15 min to separate the liquid extract from the solid residue. The liquid supernatant was transferred into vials and stored at 2^0c for phytochemical analysis (Harborne JB. Phytochemical Method: A Guide to Modern Techniques of Plants Analysis. New York: Chapman and Hall).

Pharmacological evaluation

Healthy adult male sprague-dawley (SD) rats (160 to 250 gm) were selected for the present study. The animals had free access to standard rat pellet diet, with water supplied *ad* libitum. all rats were housed in poly propylene cages at room temperature:(25 ± 2^0ċ), humidity (60±10%) with 12 hours light and dark cycle under strict hygienic conditions. The experimental protocol was approved by IAEC (institutional animal ethics committee) reg. No: 1048/po/re/s/07/CPCSEA) of chalapathi institute of pharmaceutical sciences. The study followed all the rules of committee for purpose of control and supervision of experiments on animals (CPCSEA). All procedures were carried out in accordance with the conventional guidelines for experimentation with animals [17,18]. Prior to experimental treatments, animals were fasted over night but were allowed free access to water. Five animals were used for each group of study.

Chemicals and Drugs

Source: chalapathi institute of pharmaceutical sciences, Guntur, Andhra Predesh, India. n-hexane, distilled water, concentratedHcl, 5-fluorouracil, ethanol, ethylene diaminetetraaceticacid, benzene solution (chloroform in water/2-propanol [50/50] (v/v), etc.

Benzene Induced Acute Leukemia (In Vivo Animal Model)

According to experimental procedures Leukemia was successfully induced in SD male rats by intravenous injection of 0.2 ml of a 1:10 diluted benzene solution.(chloroform in water/2-propanol [50/50] v/v), given every 2 days for 3 consecutive weeks [19,20].

The Plant extracts of Garlic and Grapes (300 mg/kg and 300 mg/kg; orally) was administered after leukemia induction. Leukemia was assessed by comparing the hematological parameters at baseline and after (Goldstein BD) leukemia induction in various experimental groups [21,22].

Blood Collection & Evaluation

After three weeks of benzene injection and treatment (as designed in the experimental protocol), animals in the respective groups were bled by cardiac puncture [23,24]. The blood was collected into ethylene di amine tetra acetic acid vials, gently mixed, labelled, and analyzed. Samples were analyzed by using Automated Haematology Analyzer to Determine Haematological Indices like white blood cells (WBCs), red blood cells (RBCs), haemoglobin, platelets, and bone marrow stem cells examination under micro scope.

Experimental Design

The rats were randomly grouped into six, with five rats in each group. Each of the rats in a group was weighed after the grouping. The standard drug and test extracts are given through orally by using gavage number 16, for two weeks, twice for a day (each 12 hr).

Group I: Normal Control.
Group II: Leukemic Control.
Group III: 5- fluorouracil (15 mg / kg) (standard drug).
Group IV: *Allium Sativum* plant extract (300 mg / kg) (Test 1).
Group V: *Vitis venifera* Plant extract (300 mg/kg) (Test 2).
Group VI: *Allium Sativum* (150 mg/kg) + *Vitisvenifera* Extracts (150 mg/kg) (total dose 300 mg/kg) (for synergistic action) (Test 3).

Major Parameters Considered For Evaluation

Base line and post analytical below mentioned parameters are evaluated [25].

- White Blood Cells (WBCs X 103 cells / µL).
- Red Blood Cells (RBCs X 103 cells / µL).
- Hemoglobin (g / dL).
- Platelets. (X 103 cells / µL).

Histomorphology of these following parameters should be analysed [26].

- Lymphocytes and myelocytes idendification in normal and leukemic bone marrow.

Signs and Symptoms Observed During The Study [27].

- Immunological Reactions: Redness of Skin and skin rashes (Present or not).
- Body Weight (Loss or gain).
- Liver and spleen (enlarged or not).

RESULTS AND DISCUSSION

Phytochemical analysis

Table-1: Phytoconstituents Present in Plant Extracts of *Allium Sativum* and Vitis*Venifera.* Absent (-), present (+), highly Present (++)

S.NO	PHYTOCONSTITUENTS		*Allium Sativum*	*Vitis Vinifera*
01.	CARBOHYDRATES	a) Fehling's Test:	+	+
		b) Molisch's Test:	_	+ +
		c) Benedict's Test:	+ +	+
02.	PROTEINS	a) Biuret Test:	+ +	+
		b) Ninhydrin Test:	+ +	+ +
		c) Xanthoprotein Test:	+ +	_
03.	ALKALOIDS	a) Mayer's Test:	+ +	_
		b) Dragendroffs Test:	_	+ +
		c) Wagner's Test:	+ +	+
04.	GLYCOSIDES	a) Brontragers's Test:	+	_
		b) Modified Brontragers Test:	+ +	+ +
05.	SAPONINS	a) Foam Test:	+ +	+
06.	TANNINS	a)Ferric chloride Test:	+	+ +
		b) Lead Acetate Test:	+ +	+
07.	FLAVONOIDS	a) Aluminium chloride test	+ +	+ +
08.	POLY PHENOLICS	a) Folin-ciocalteau reagent	+ +	+ +

Spectral analysis

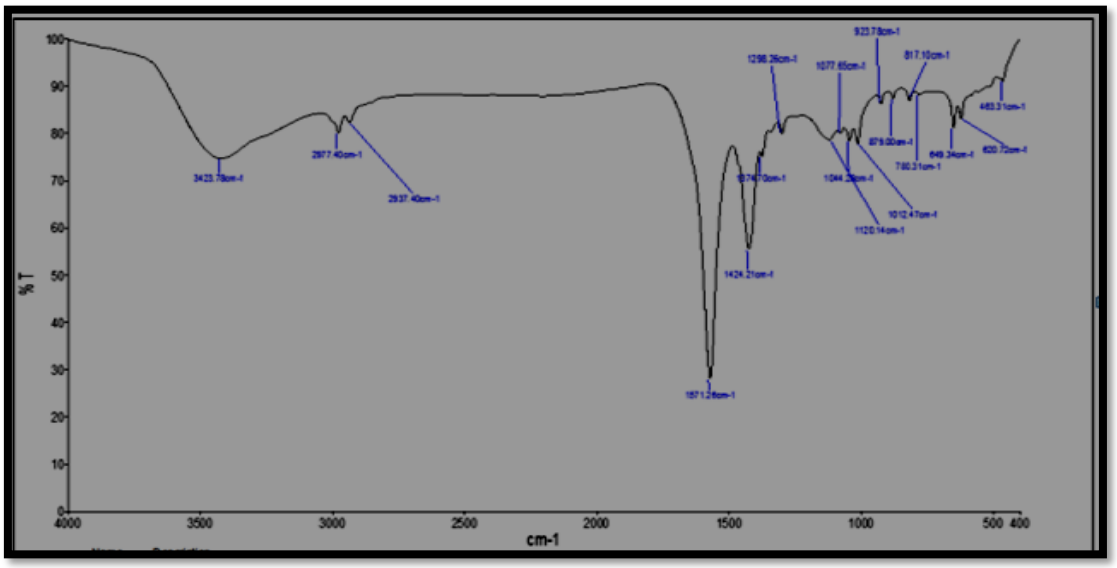

Fig-1: IR Spectral Analysis of *Allium Sativum*

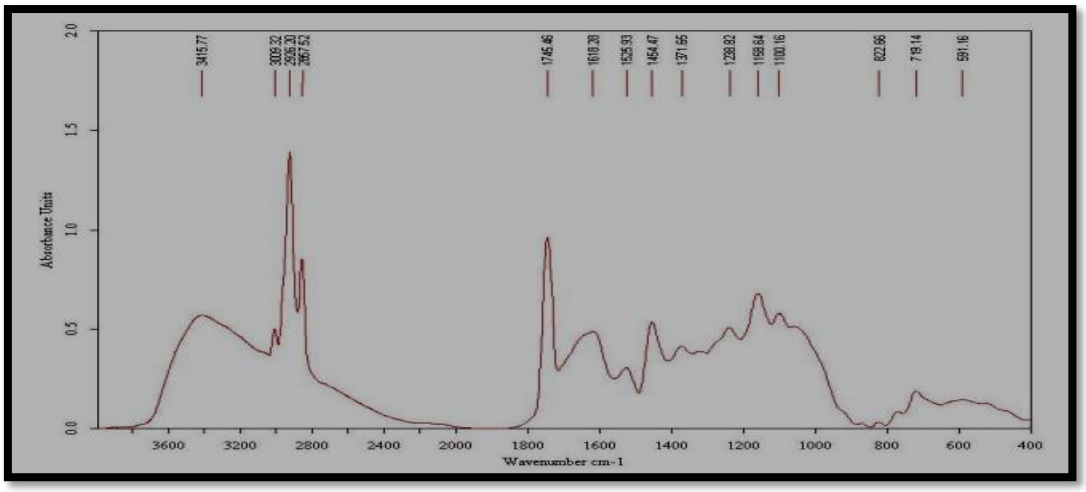

Fig-2: IR Spectral Analysis of VitisVenifera

Table-04:Comparision of Base Line and Post Analytical Blood Parameters

Groups	Sample	WBC (X 103 cells / µL)	RBC (X103cells / µL)	Hgb (g /dL)	Platelets (X 103 cells/ µL)
I (normal control)	Base line	8.28±0.65	6.62±0.48	12.18±0.70	326.14±24.56
	Post analytical	9.02±0.28	6.84±0.58	12.58±0.56	342.20±26.68
II (leukemia control)	Base line	9.12±0.90	6.58±0.56	12.26±0.38	334.80±34.96
	Post analytical	14.12±0.58	4.08±0.88	8.56±0.78	422.36±28.24
III (5- fluorouracil (15 mg/kg))	Base line	13.26±0.60	6.50±0.46	11.98±0.44	326.26±24.22
	Post analytical	10.12±0.28	5.90±0.26	10.64±0.78	302.56±20.90
IV (sample extract 1 (300 mg/kg))	Base line	13.16±0.52	7.02±0.58	12.20±0.18	332.74±26.37
	Post analytical	12.25±0.62	7.12±0.64	11.58±0.46	402.56±24.62
V (sample extract 2(300 mg/kg))	Base line	13.52±0.36	7.18±0.86	12.56±0.70	320.48±20.28
	Post analytical	12.02.±0.72	7.48±0.38	12.08±0.18	420.74±26.32
VI (sample extracts one and two) (300mg/kg) synergistic action	Base line	13.32±0.48	6.82±0.26	12.08±0.16	346.12±24.16
	Post analytical	11.12±0.52	6.25±0.16	11.04±0.26	328.26±20.94

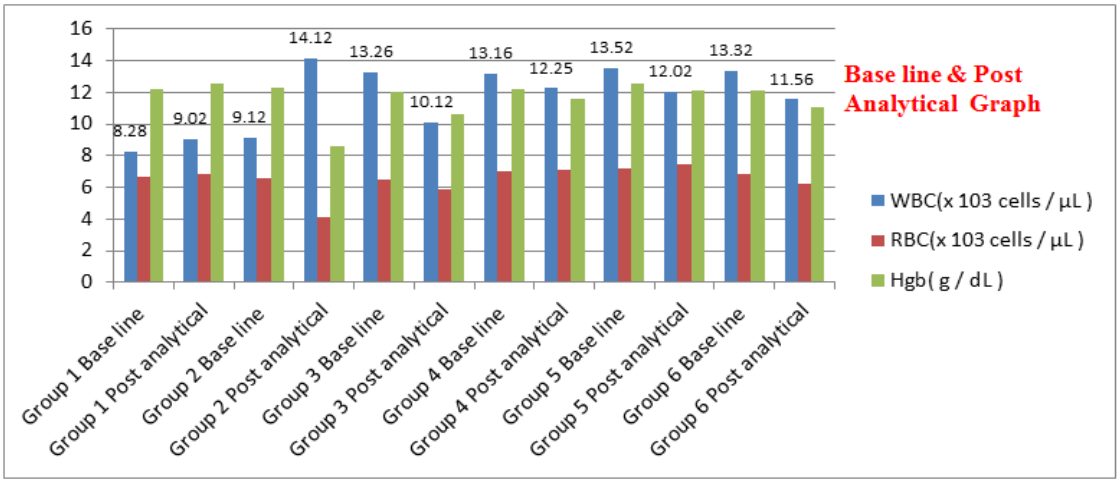

Fig-4: Represents the Comparision of Base Line and Post Analytical Blood Parameters

Values are expressed as mean ± SEM of five animals in each group, normal control group compared with treated groups. Significance at $P < 0.05$.

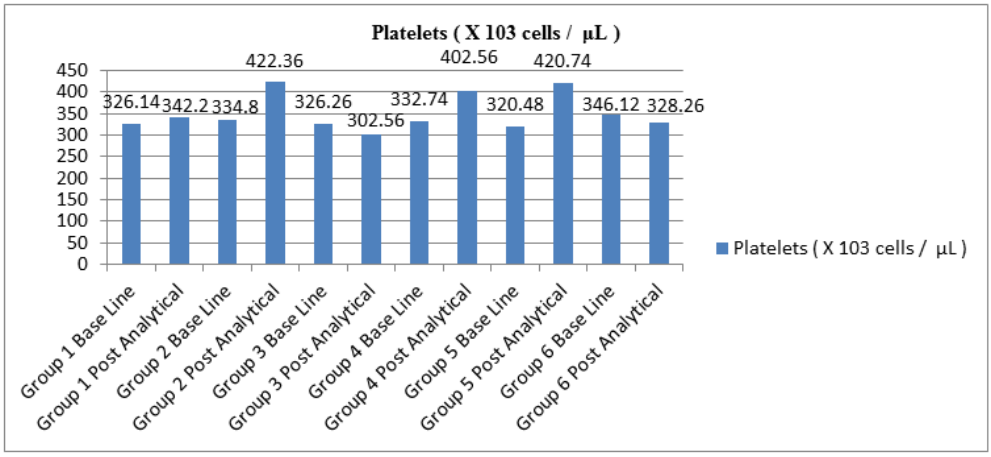

Fig-5: Represents the Comparison of Base Line and Post Analytical Data of Platelets

Values are expressed as mean ± SEM of five animals in each group, normal control group compared with treated groups Significance at P < 0.05.

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Histomorphology Reports

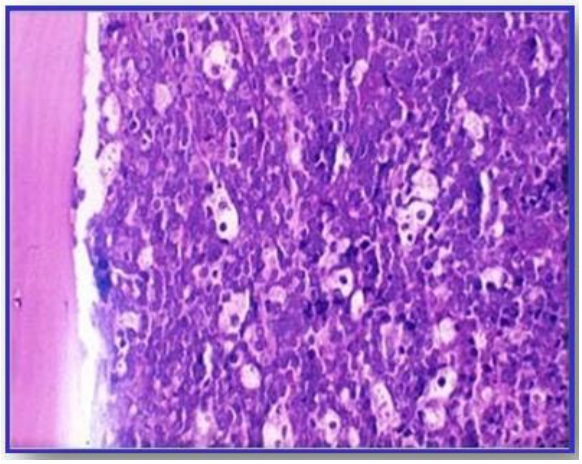

Fig-6: Normal bone marrow

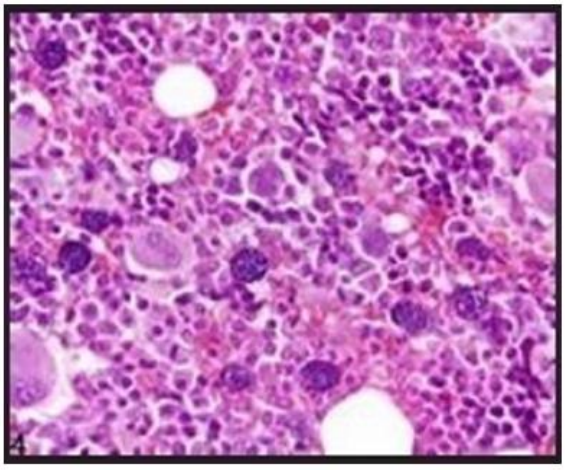

Fig-7: Acute Leukemic Bone Marrow

Reports from Signs and Symptoms

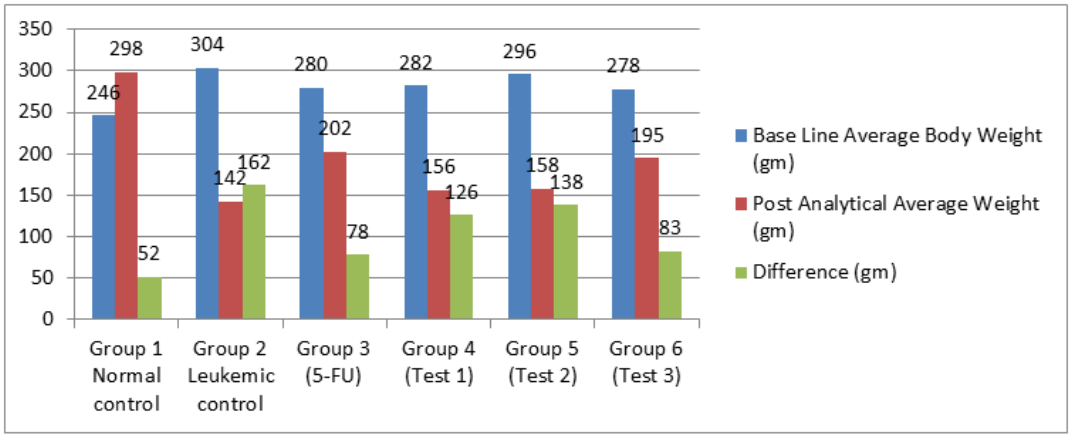

Fig-8: Represents the Difference between Base Line & Post Analytical Body Weights (gm)

Values are expressed as mean ± SEM of five animals in each group, normal control group compared with treated groups. Significance at P < 0.05

Spleen and Liver

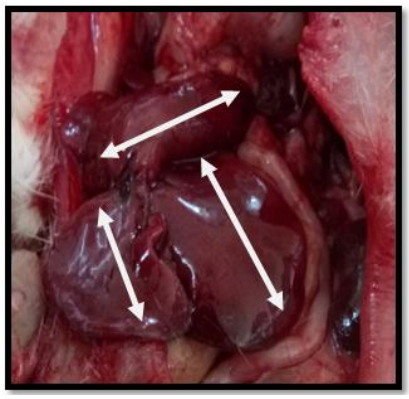

Fig-9: Normal liver

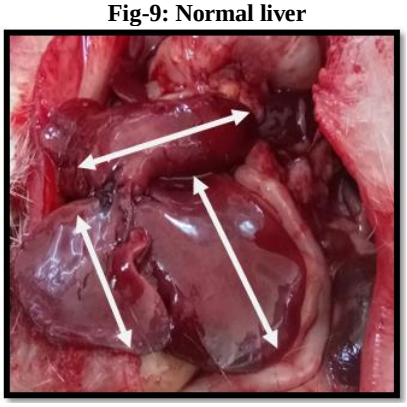

Fig-10: Enlarged liver

Fig-11: Normal spleen

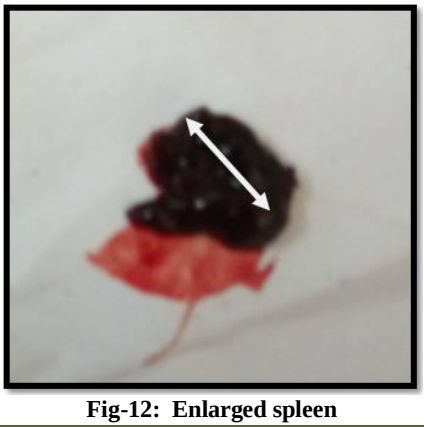

Fig-12: Enlarged spleen

Liver and spleen parameters says

- The normal control group liver weight = 8.12±1.6 gm.
- The leukemic control group liver weight =12.52±1.4 gm.
- The normal control group spleen weight = 0.82±0.12 gm.
- The leukemic control group spleen weight = 1.02±0.25 gm.

Immunological Reactions Observed

- Redness of skin at neck region (fig.no:13).
- Redness of skin at face and neck regions (fig.no:14).

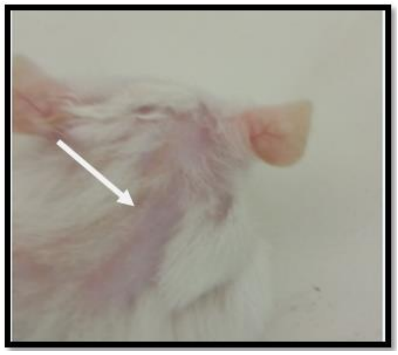

Fig-13: Skin redness and rashes

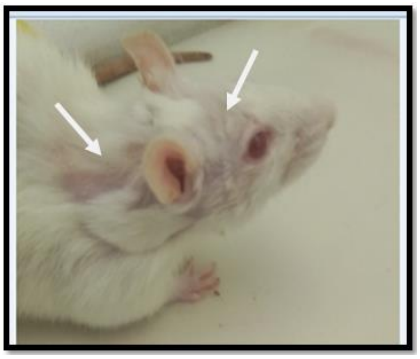

Fig-14: Skin redness and rashes

DISCUSSION

Blood Evaluation Parameters Says

The in vivo anti leukemic effect was evaluated on benzene induced acute leukemic SD rats (benzene was chosen to induce leukemia in murine model). Animals were treated with n-hexane extract of *Allium sativum* (group 4 its considered as a test 1) (300 mg/kg), aqueous extract of *vitis venifera* (group 5 its considered as a test 2) (300 mg /kg), both extracts of test one and test two (synergistic action considered as a test 3) (300 mg/kg) are compared with standard drug treatment (5-fluorouracil for group 3) (15 mg/ kg) and disease control, normal control groups.

According to base line and post analytical graph (fig.no:4) in leukemic control group (group 2) shows higher white blood cell count due acute leukemia compare to other groups. After treatment with test 1, test 2, test 3,and standard drug (5-fluoro uracil),its shows reducing count of white blood cells in all treatment groups. Why because the treatment was targeted to promote apotosis of leukemic blast cells and kill the leukemic blast cells in both test and standard treatment groups [28]. But group 6 blood samples shows more reducing count of white blood cells compare to group 4 and group 5 but not more then or equal to standard drug treatment group. its indicates that the *Allium sativum* and *Vitis venifera* extracts shows synergistic action on group six animals. Platelet number is also altered during leukemia (fig.no:5). There is also impairment in release of normal platelets during leukemia.

Significantly increased platelets count in posttreatment groups of group four and group five but not in group 3 and group 6. Its indicates the individual extracts of test one and two does not more effective to increasing of the healthy white blood cells during in acute leukemia treatment but its increase platelets count.Coming to RBC count and heamoglobulin, its not much effected due to benzene induced acute leukemia on SD rats. But finally stated that leukemia is observed by an increased number of abnormal WBC (blast cells) then normal count (fig.no.4).

Histomorphology Says

Reports from the histomorphology of normal control (group 1) and leukemic control (group 2) bone marrow (fig.no.6 & 7) examination says that compare to normal control bone marrow the leukemic control bone marrow shows more number of immature WBC (blast cells) its indicates acute leukemia.

Signs and Symptomes Says

Reports from the signs and symptoms it shows signs and symptoms like weight loss (fig.no:8), liver and spleen enlargement (fig.no:10 & 12), and skin allergic reactions like redness of the neck and face surface was observed (fig.no:13 & 14) mostly in leukemic control group (group 2) and also observed little bit in both test and standard drug treatment groups.

CONCLUSION

The current work stats that, the combination of plant extracts of *Allium sativum* and *Vitis venifera* shows synergistic action on group six animals. It was increase their efficacy then individual plant extracts on benzene induced acuteleukemic male sprague - dawley (SD) rats. According to this study reports the both extracts of *Allium sativum* and *Vits venifera* its not only for acute leukemia but also its shows immunomodulatory action and also increasing the count of platelets in benzene induced acute leukemic male sprague–dawley (SD) rats. It demonstrated anti leukemic potential that might be due to the presence of alkaloids, glycosides, tannins, Polyphenolics and flavonoides in both plant extracts.

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Conflicts of interest

The authors declare that there is no conflicts of interest.

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Abbreviations

AML, acute myeloid leukemia ; ALL, acute lymphoid leukemia ;DNA, deoxy ribonucleic acid; Hcl, hydrochloric acid; Rpm, revolutions per minute; SD, Sprague-dawley; IAEC, institutional animal ethics committee; CPCSEA, committee for purpose of control and supervision of experiments on animals; 5-FU, 5-fluorouracil; WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin; Plt, platelets; AHA, Automated Hematology Analyzer;ANOVA, analysis of variance; IR, infra red spectroscopy;SEM, standard error of mean.