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In-vivo and In-vitro Evaluation of Anticancer Activity of Snuhi Kshara using Dalton's Lymphoma Cells

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ABSTRACT

Although, *kshara* therapy is reported to be one among the possible measures in the treatment of *arbuda* in Ayurvedic science, a limited individual practitioners are practicing it. This mode of treatment has been proved to terminate the invasion of fast growing tumour cells and clinically shown to render significant symptomatic relief. But still the *kshara* applications are rarely tested internally. The *kshara* variety meant for internal application, termed as *paneeyakshara*, is prepared by dissolving *kshara* in water. *Sushruta* has described the fact that *paneeyakshara* has a greater affinity for the destruction of new tissues. Based on this finding, it has been proposed to be useful for the destruction of unwanted tissues. Furthermore, the exploration of new plants for the preparation of *Kshara* as better alternates for existing medicines is very much needed. In the present work, Snuhi, *Euphorbia nerifolia,* has been opted for preparation of *kshara*. Snuhi *kshara* at different concentrations have shown dose dependent inhibition of SKMEL cancer cells in the MTT assay with an IC_{50} value of 84.36 µg/mL. and the life extent of tumour induced mice increased to 74%, 69% and 77% respectively with the concentration of 200, 100 and 400 mg/Kg of test drug. In the DLA tumour bearing mice, the average life span was recorded to be 54. The improvements in biochemical parameters with the test drug at dosage of 400 mg/Kg was almost comparable with that of the standard drug 5-fluorouracil, restates the anticancer potential of Snuhi *kshara*.

INTRODUCTION

Kshara therapy or alkaline therapy is meant to treat diseases that cannot be cured by other modes. *Kshara* preparation involves the extraction of alkali from the ash of dried plants. The term *Kshara* has found its origin from the two roots, *Chhana* and *Chhar* that means Satan or Destroyer as it can destroy unwanted tissues in the body [1]. Acharya *Sushruta* has described *Kshara* as having *Ksharana* and *Kshanan* properties, that is it can melt away or destroy lesions. While, *Sushruta* mentions *Kshara* (caustics) as Pradhana (great), *Dalhanacharya*, the commentator of *Sushruta Samhita*, specifically mentions that *Jalauka* is *Pradhana*, *Agni* is *Pradhantara* (better) and *Kshara* (caustics) is *Pradhanatama* (best). He explained as the *Kshara Pradhanatamata* is due to the fact that *Agni* and *Jalauka* cannot act as *Chedana* (excision) while *Kshara* acts as *Chedana*. Due to its properties, *Ksharak*arma cannot be used in Pittaja disease but *Dalhanacharya* mentioned its use in *Pittaja Arsh*. He also gave the logical interpretation for acceptance of *Kshar Pradhanata* by specifically differentiating *Ksharana* and *Kshanana*. "*Ksharanata*" "*dushtatwakmansadi chalanat shatanata*" that means cutting, and "*kshananata twakmanasadi Hinasanaat*" i.e *Kshanana* means to dissolve. Thus *Dalhanacharya* described the perfect mode of action of *Ksharakarma* [2, 3]. Modern Ayurvedic literatures also consider *Kshara* as alkali having hot, piercing and scraping properties. *Kshara* is considered to be an important *Anushashtra* (minimal surgical access) as it can carry out excision, incision and scrapping simultaneously.

According to *Sushruta* Samhita, plants containing *kshara* were collected, dried and burnt to collect the ash. The burning should be done until all the matter get burnt. The ash thus obtained need to be dissolved in six times of water in an earthen pot and kept overnight. All the contents should be filtered 21 times the next day morning and the liquid thus obtained to be heated for evaporating the water content and *kshara* will be obtained [4]. As per *Sharangdhara Samhita*, *kshara* is obtained by burning parts of plants exuding milky sap in fire. Thus formed ash will be dissolved in four times of water in mud pot and will be kept overnight. Next day morning, the clear supernatant will be decanted from the suspension into a clean vessel and to be boiled for removing all the water content. Finally, a fine white powder will be left at the bottom, known as *kshara* [5]. The *kshara* obtained from plant sources are classified as *Vanaspatijanya*. *Apamarga kshara*, *Yava kshara* are some examples for *Vanaspatijanya kshara*. Based on their mode of application, *kshara* are classified into *Pratisaraniya* and *Paneeya kshara*. *Pratisaraniya kshara* is used for external application while *Paneeya kshara* is used internally.

Snuhi Panchang is of having high therapeutic potential and it has been used in the formulation of *Kshara sutra*. Primarily, the *kshara* prepared from *Euphorbia neriifolia* has been applied in the form of medicated thread for treating piles and fistula[6]. A multicentric randomized controlled trial carried out by Indian Council of Medical Research established that the long term out come with *Kshara sutra* was superior to the surgery, offering an active and safe mode of management for patients with fistula-in-ano. Snuhi *kshara* (SK) was prepared by processing the ash of *Snuhi Panchang*. Euphol is the chemical compound present in *Euphorbia neriifolia* [7]. SK is described to be useful for the cure of sthoulya (obesity). The *kshara* helps in removing vitiated *Kapha, Medas* and *Kleda* from the body and their by helping to decrease the weight [8]. The *kshara* has been reported to be used for external applications in cancer therapy and need to be studied further for internal applications. However, no reports are available proving the anticancer property of Snuhi *kshara*. Since the study has not

been done in any manner previously, an experimental study was conducted before clinical trial to record toxicity, to find out an animal dose and to propose a dose for clinical setting for solid tumors. Thus the aim of present study is to assess the anticancer activity of the test drug *Snuhi Paneeya kshara* against DLA induced solid tumors in experimental mice.

Experimental Section

Preparation of *Snuhi kshara*

SK has been prepared by following method mentioned in the text *Ayurveda Sara Samgraha* with modification in soaking duration at the Postgraduate Department of *Dravyaguna Vigyan*, Mannam Ayurveda Co-operative Medical College, Pandalam, Pathanamthitta, Kerala [9].The plant was cut into small parts and dried in sunlight. Dried plant was subjected to closed-burning (*anthardhuma*) in a big iron pan till it was converted into total ash. After self-cooling, greyish white ash was collected. The prepared ash and R.O. (Reverse osmosis) water were taken in cylindrical S.S. vessel with ratio of 1:8 (v/v). The mixture was macerated thoroughly with hands and kept undisturbed for overnight soaking. The next morning, clear supernatant liquid was decanted carefully and filtered seven times through four folded cotton cloth to ensure that all dissolvable alkaline particles were fully extracted. The supernatant particles of charcoal and the solid residue were eliminated. The obtained filtrate (*Ksharajala*) was subjected to heat at 98 ℃ to evaporate the water content. After complete evaporation of water portion, the obtained *Kshara* was collected from the inner surface of S.S. vessel by scraping and stored in an air tight glass container. Care should be taken that the *kshara* obtained was not contaminated or exposed to moisture, as it is more liable to liquidate because of its hygroscopic nature being an organic salt, when exposed.

Evaluation of Anticancer Activity of Snuhi *Kshara* using Dalton's Lymphoma Cells

SKMEL cancer cells (2500 cells/well) were seeded on 96-well plates and allowed to acclimatize to the culture conditions such as 37 ˚C and 5% CO_2 environment in the incubator for 24 h. The test samples were prepared in DMEM media (100 mg/mL) and filter sterilized using 0.2 µm Millipore syringe filter. The samples were then diluted in DMEM media and poured to the wells containing cultured cells at final concentrations of 6.25, 12.5, 25, 50, 100 µg/mL respectively. Untreated wells were kept as control. All the experiments were done in triplicate and average values were taken to minimize errors. After treating with test samples, the plates were incubated for further 24 h followed by media discarded. 100 µL of 0.5 mg/mL MTT solution in PBS was added to the wells. The plates were further incubated for 2 h for the formation of formazan crystals. The supernatant was discarded and 100 µL DMSO (100%) were added per well. The absorbance at 570 nm was quantified in a micro plate reader. Two wells per plate without cells served as blank. All experiments were done in triplicates. The cell viability was expressed using the following formula:

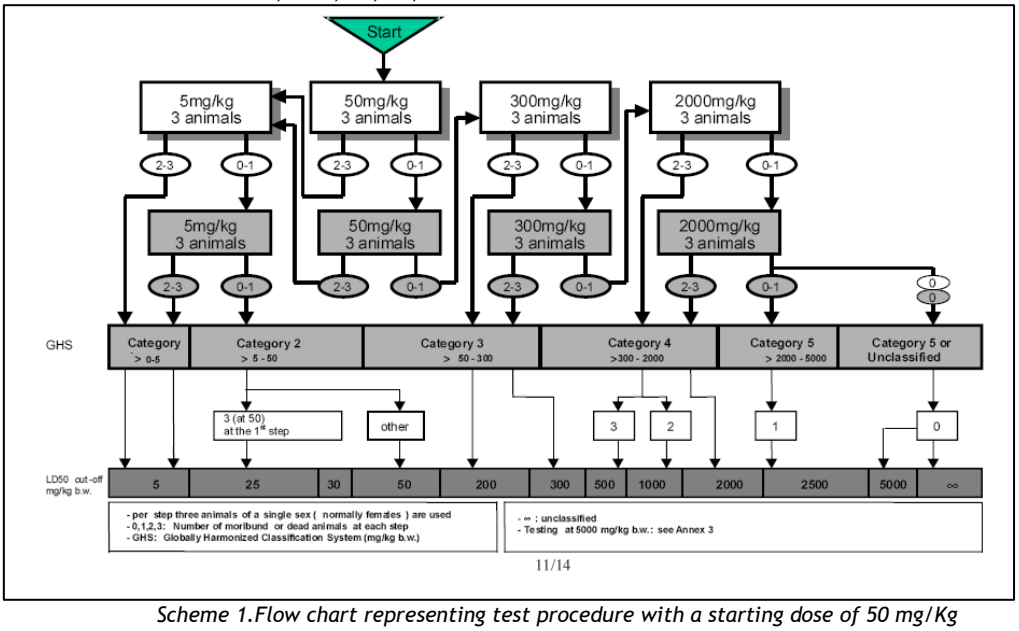
$$\textit{Percentage of cell viability} = \frac{\textit{Average absorbance of treated}}{\textit{Average absorbance of control}} \times 100$$

Experimental Animals

Healthy Swiss Albino female mice, weighing about 150-200 gm were obtained from the animal house. This facility was approved by the Institutional Animal Ethical Committee (IAEC) which was certified by the Committee for the purpose of control and supervision of experiments on animals.

The animals were housed in clean and dry polycarbonate cages and kept in a well-ventilated animal facility with 12-hour light and 12-hour dark cycle. They were fed a standard pellet diet for 7 days and had free access to water. For the experiment, the animals were fasted overnight but had access to water. After fasting, they were administered the test drug orally at a dose of 2000 mg/Kg body weight. This starting dose was chosen because most test drugs have an LD_{50} greater than 2000 mg/Kg body weight.

Following oral administration, the mice were monitored hourly for 24 hours to assess mortality and observe any changes in the autonomic or behavioural responses viz. alertness, spontaneous activity, salivation, respiration, urination, aggression, irritability, convulsions and corneal reflex. The mice were observed regularly for 14 days to record any signs of mortality or toxicity. Since no deaths were observed according to the guidelines, the study was repeated with the same dose to confirm the findings. The flow chart illustrate the procedure used for this method.



Scheme 1.Flow chart representing test procedure with a starting dose of 50 mg/Kg

Evaluation of Anticancer Activity in DLA induced mice

Dalton's Lymphoma Ascites (DLA) cells were supplied by Amala Cancer Research Centre in Trissur, Kerala, India. These cells were maintained *in-vivo* in Swiss albino mice through intraperitoneal transplantation. To transfer the tumour cells to the experimental animals, DLA cells were aspirated from the peritoneal cavity of the mice using saline. Cell counts were performed and the cells were diluted to achieve a total concentration of 1 x 10^6 cells per mouse. This dilution was then administered intraperitoneally. Tumour growth was allowed to proceed for at least seven days before beginning treatments.

Swiss Albino mice were divided into six groups, with each group containing six mice each. Five of these groups were injected intraperitoneally with DLA cells (1 × 10^6 cells per mouse), while the remaining group served as normal control group.

Table 1. Treatments received for each group of animals

G_1	Served as the normal control. Received normal diet and Water
G_2	Served as the tumour control. Fed with normal diet and Water
G_3	Served as the positive control. Treated with fluorouracil injection at 20 mg/Kg body weight, intraperitoneally.
G_4	Served as a low dose treatment control. Administered test drug 200 mg/Kg *Snuhi paneeya kshara* W.S.R.
G_5	Served as a low dose treatment control. Administered test drug 100 mg/Kg *Snuhi paneeya kshara* W.S.R.
G_6	Served as a high dose treatment control. Administered test drug 400 mg/Kg *Snuhi paneeya kshara* W.S.R.

In this study, drug treatment was given after the 24 hrs of injection, once daily for 21 days. On day 21, after 24 hrs the last dose, all mice from each set were forewent; blood was withdrawn from each mouse by cardiac puncture method and the following parameters were checked.

Haematological parameters

- White blood cells (WBC)
- Red blood cells (RBC)
- Haemoglobin content (Hb)
- Platelet count
- Packed cell volume (PCV)

Serum enzyme and lipid profiles

- Total cholesterol (TC)
- Triglycerides (TG)
- Aspartate amino Transferase (AST)
- Alanine amino Transferase (ALT)
- Alkaline Phosphatase (ALP)

Derived parameters

- Body Weight: The body weight of all the mice was recorded from the start of the study to the 15th day. The average increase in body weight on the 15th day was calculated.
- Life Span (%): The percentage increase in life span (% ILS) was determined using the following formula

$$\%\ \text{ILS} = \frac{Life\ span\ of\ treated\ group}{Life\ span\ of\ control\ group} - 1 \times 100$$

- Cancer Cell Count: A 0.1 mL sample of fluid was extracted from the peritoneal cavity of each mouse using a sterile syringe. This sample was diluted with 0.8 mL of ice-cold normal saline or sterile Phosphate Buffer Solution and mixed with 0.1 mL of trypan blue (0.1 mg/mL). The total number of the viable cells was counted using haemocytometer.

$$\text{Cell count} = \frac{\text{Number of cells} \times \text{Dilution}}{\text{Area} \times \text{Thickness of liquid film}}$$

Results and Discussion

Evaluation of Anticancer activity of Snuhi *kshara* by MTT Assay

The *in-vitro* anticancer activity of Snuhi *kshara* was determined using MTT assay against SKMEL cancer cells to measure cell viability.

Table 2. Evaluation of Anticancer activity of Snuhi *kshara by MTT Assay*

Samples	Triplicate 1	Triplicate 2	Triplicate 3	Average
Control	0.678	0.672	0.657	0.669
6.25	0.634	0.626	0.618	0.626
12.5	0.585	0.572	0.566	0.574
25	0.496	0.487	0.478	0.487
50	0.396	0.417	0.408	0.407
100	0.299	0.31	0.316	0.308
Concentration (µg/mL)	**Percentage of viability**			**IC_{50}**
6.25	93.57			**84.36**
12.5	85.85			
25	72.80			
50	60.84			
100	46.09			

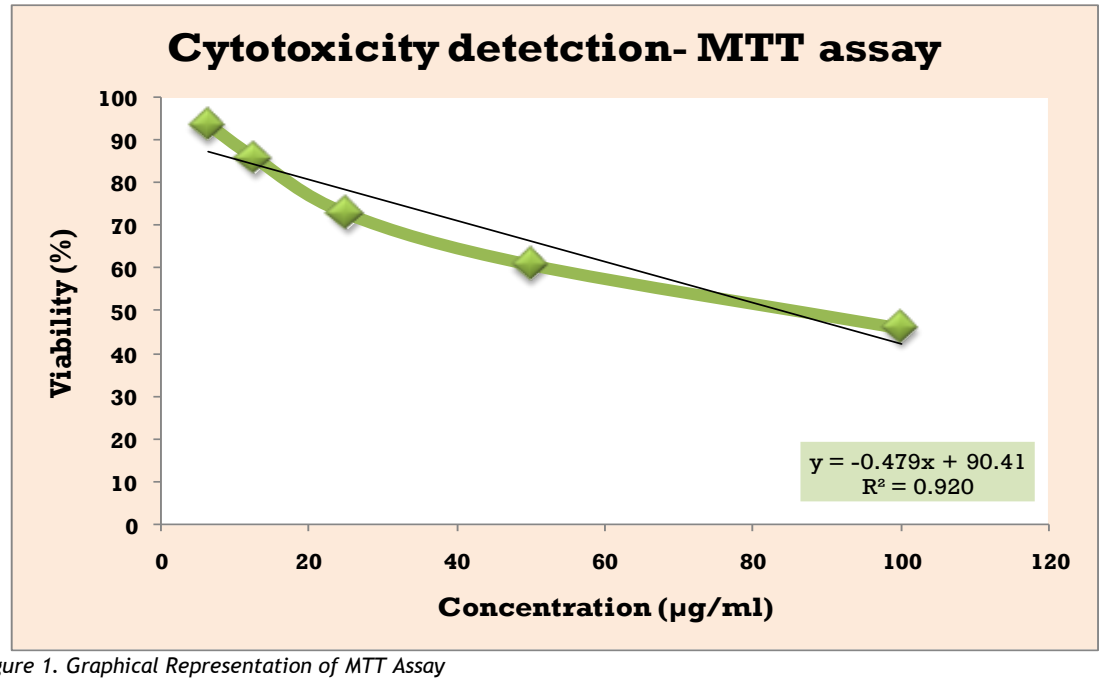


Figure 1. Graphical Representation of MTT Assay

Dose dependent reduction in cell viability was observed in SKMEL cancer cells administered with different concentrations of the snuhi *kshara* sample, **SK**. The **IC_{50} value** was obtained as **84.36 µg/mL** for the sample.

DLA induced Anticancer study of Snuhi *Kshara* (SK)

Acute Toxicity Study

During the acute toxicity study, the test drug was administered orally and animals were observed for mortality, changes in the autonomic nervous system, central nervous system and behavioural responses. There was no mortality observed even at 2000 mg/Kg for the test drug. All the animals were found to be normal and there were no behavioural changes till the end of the observation period. This observation revealed that the test drug is very safe up to 2000 mg/Kg of body weight known as maximum tolerated dose (MTD) by acute toxicity model study as per OECD guidelines 423. Hence from this 1/10th and 1/5th of MTD was selected and the effective doses were stayed fixed as 200 and 400 mg/Kg for further pharmacological studies.

Effect on Haematological Parameters

WBC

Table 3. WBC count in treatment groups

GROUP	MEAN ± SD	F VALUE	p VALUE	SIGNIFICANCE
G_1	8.147 ± 0.593	16.435	< 0.05	YES
G_2	12.681 ± 1.415			
G_3	11.101 ± 1.055			
G_4	13.147 ± 1.5			
G_5	12.558 ± 0.791			
G_6	11.105 ± 1.001			

Where G_1- Normal control, G_2 - Tumour control, Group 1 and 2 receives normal diet and Water. G_3 - Positive control (fluorouracil at 20 mg/kg), G_4-Treatment control (test drug 200 mg/kg), G_5 - Treatment control (test drug 100 mg/kg), G_6 - Treatment control (test drug 400 mg/kg).

The mean and standard deviations of the six groups are shown in the table. For comparing these groups, we use One- way Anova test. At 5% level of significance, the test has shown great significantdifference, as the p-value obtained is less than 0.05. The mean values are represented graphically using a line graph. From the results obtained it is evident the test drug at a concentration of 400 mg/Kg is as effective as the standard drug, fluorouracil.

Effect on Tumour Growth

The effect of test drug on tumour growth responses were observed and shown in Table. In the DLA tumour control group, the average life span of animals stood found to be 54% whereas, 200, 100 and 400 mg/Kg of test drug showed increase in life span to 74%, 69% and 77% respectively. These values were significant ($p < 0.001$) when compared with cancer control group mice. The average life span of 5- FU treated was found to be 90%, indicating its potent antitumor nature. The antitumor nature of test drug was evidenced by the significant ($p < 0.01$, $p < 0.001$) reduction of increase in body weight in animals treated with test drug at the dose of 200, 100 and 400 mg/kg test drug when compared to DLA tumour bearing mice. There was a significant ($p < 0.001$) reduction in packed cell volume and viable tumour cell count were found with 200, 100 and 400 mg/kg of test drug when compared to the DLA tumour control.

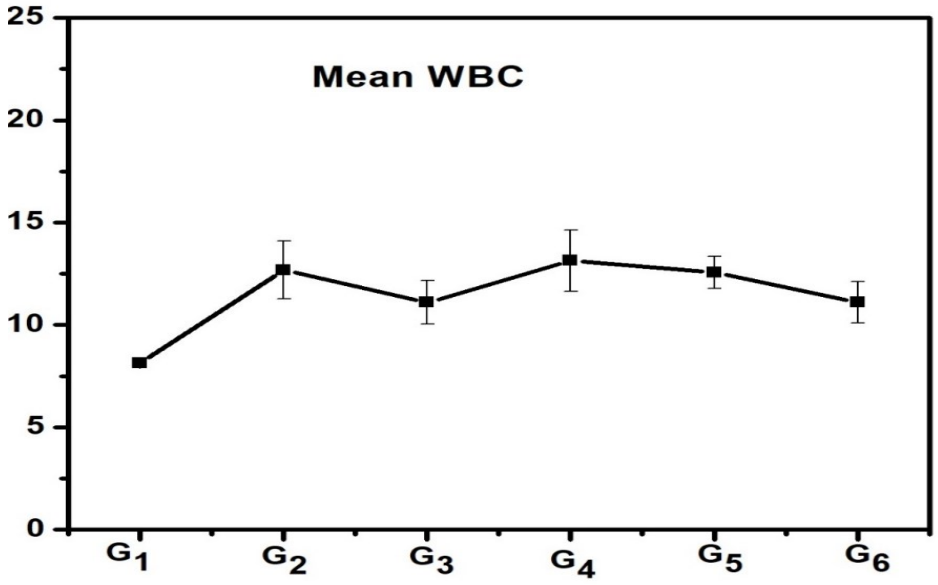


Figure 2. Mean WBC in different Treatment Groups

RBC

Table 4. RBC WBC count in treatment groups

GROUP	MEAN ± SD	F VALUE	p VALUE	SIGNIFICANCE
G_1	3.483 ± 1.062	6.937	< 0.05	YES
G_2	1.271 ± 0.275			
G_3	2.9 ± 0.463			
G_4	2.847 ± 0.699			
G_5	2.677 ± 0.605			
G_6	2.348 ± 0.773			

The mean and standard deviations of the six groups are shown in the table. For comparing thesegroups, we use One- way Anova test. At 5% level of significance, the test has shown great significant difference, as the p-value obtained is less than 0.05.

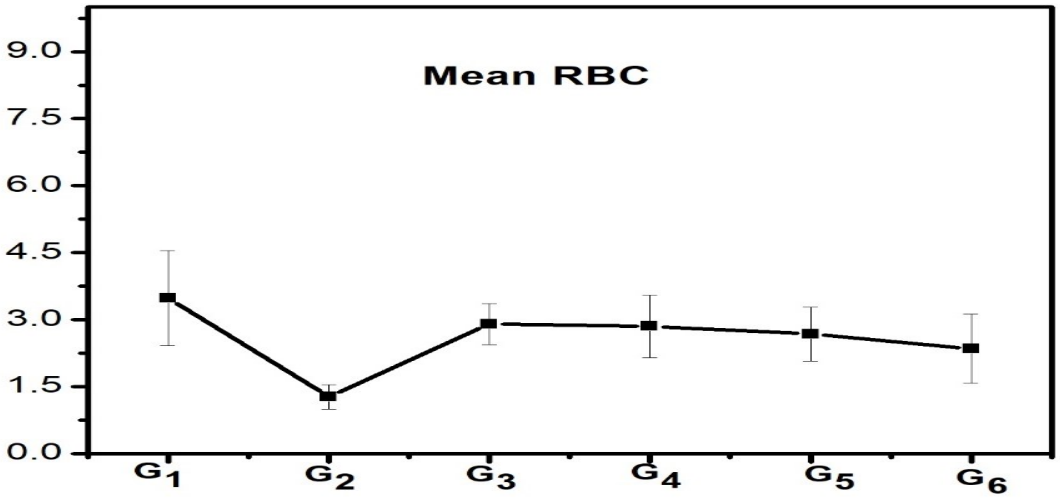


Figure 3. Mean RBC in Different Treatment Groups

The mean values are represented graphically using a line graph. From this, it is clear that the mean RBC of all the treatment control groups are more or less same as compared to that of the standard drug. Hence the drug is almost as efficient as the standard drug.

Haemoglobin (Hb)

Table 5. Hb Count in Different Treatment Groups

GROUP	MEAN ± SD	F VALUE	p VALUE	SIGNIFICANCE
G_1	13.513 ± 1.021	48.576	< 0.05	YES
G_2	6.587 ± 0.677			
G_3	11.45 ± 0.426			
G_4	9.203 ± 0.966			
G_5	8.978 ± 0.528			
G_6	10.805 ± 1.146			

The mean and standard deviations of the six groups are shown in the table. For comparing thesegroups, we use One- way Anova test. At 5% level of significance, the test has shown great significant difference, as the p-value obtained is less than 0.05. From this, it is clear that the mean Hb of the treatment control groups are found to be less than that of the standard group, but higher than the tumor group. At a higher concentration, the test drug is showing a comparative effect as that of the standard drug. A line graph is drawn to show the mean values.

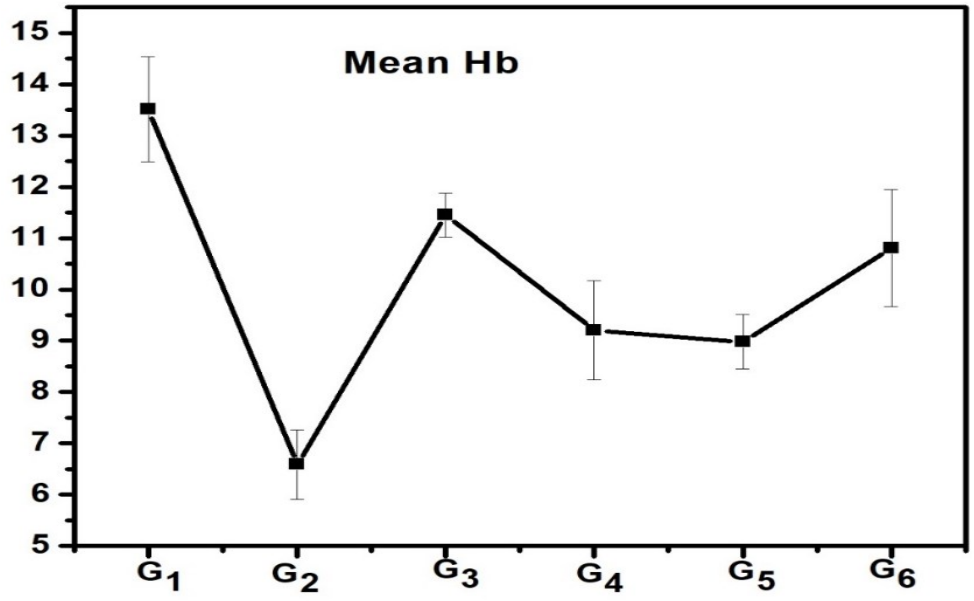


Figure 4. Mean Hb in Different Treatment Groups

Platelets

Table 6. Platelet Count in Different Treatment Groups

GROUP	MEAN ± SD	F VALUE	p VALUE	SIGNIFICANCE
G_1	3.452 ± 0.825	6.404	< 0.05	YES
G_2	2.25 ± 1.089			
G_3	2.195 ± 0.903			
G_4	1.25 ± 0.402			
G_5	1.102 ± 0.042			

G_6	2.307 ± 1.106			

The mean and standard deviations of the six groups are shown in the table. For comparing thesegroups, we use One-way Anova test. At 5% level of significance, the test has shown great significant difference, as the p-value obtained is less than 0.05.

From this, it is clear that the mean of platelets of the treatment control group 1 and 2 are less than positive control group, but TC 3 shows a greater value as compared to the standard drug. A line graph is drawn to show the mean values.

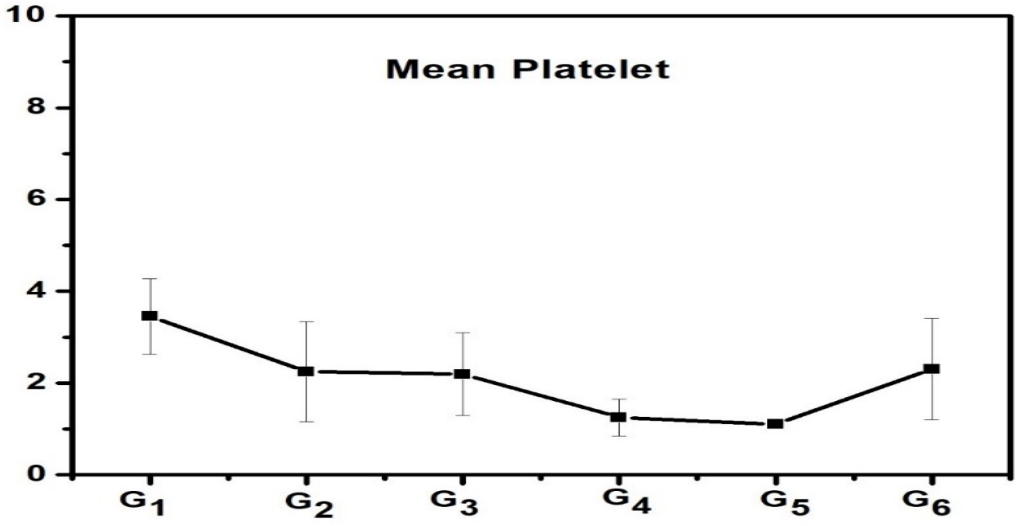


Figure 5. Mean Platelet Count in Different Treatment Groups

The comparative effects of the test drug on various haematological parameters in cancer-bearing mice are summarized in the table below. In the cancer control group, RBC count, haemoglobin (Hb) content and platelet count were significantly ($p < 0.001$) decreased. These parameters returned to normal levels following treatment with the test drug at doses of 200, 100 and 400 mg/Kg. Additionally, the white blood cell (WBC) count, which was significantly elevated ($p < 0.001$) in the DLA control group was normalized with the test drug at the doses of 200, 100 and 400 mg/Kg. However, the standard treatment, 5-FU at a dose of 20 mg/Kg body weight, yielded better results across all these parameters.

Table 7. Effect of test drug on haematological parameters

Treatment	Total WBC Cells/mLx10^3	RBC Count Mill/cumm	Hb gm/dL	Platelets Lakhs/cumm
G1	8.15 ± 0.55	3.52±1.80	13.51 ± 1.63	3.46± 0.38
G2	12.68 ±1.80^{a**}	1.27 ±0.40^{a**}	6.60 ±1.35^{a**}	1.60 ±0.56^{a**}
G3	11.10 ±1.35^{b**}	2.90±1.25^{b**}	11.45 ±1.80^{b**}	2.20±0.74^{b**}
G4	13.15±1.86^{b*}	2.85 ± 0.80^{b*}	9.20 ±1.46^{b*}	1.25±0.31^{b*}
G5	12.56±1.23 ^{b*}	2.68±0.27 ^{b*}	8.98±1.08 ^{b*}	1.10±0.65 ^{b*}
G6	11.11 ±1.03^{b**}	2.35±0.40^{b**}	10.80±1.70^{b**}	2.31 ±0.45^{b**}

All values are expressed as mean ± SEM for 6 animals in each group.
**a – Values are significantly different from the control group (G_1) at $p < 0.001$
*b – Values are significantly different from the cancer control group (G_2) at $p < 0.01$
**b – Values are significantly different from the cancer control group (G_2) at $p < 0.001$

Effect on Biochemical Parameters

Cholesterol

Table 8. Total Cholesterol in Different Treatment Groups

GROUP	MEAN ± SD	F VALUE	p VALUE	SIGNIFICANCE
G_1	106.18 ± 0.689	1026.8	< 0.05	YES
G_2	142.02 ± 0.501			
G_3	110.32 ± 0.788			
G_4	125.15 ± 1.472			
G_5	127.82 ± 0.835			
G_6	123.1 ± 1.275			

The mean and standard deviations of the six groups are shown in the table. For comparing these groups, we use One-way Anova test. At 5% level of significance, the test has shown great significant difference, as the p-value obtained is less than 0.05. The mean values are represented graphically using a line graph. From this, it is clear that the mean cholesterol of all the treatment control groups are less than that of the tumor group, but higher than the standard drug. Hence the drug is effective in reducing cholesterol count to some extent.

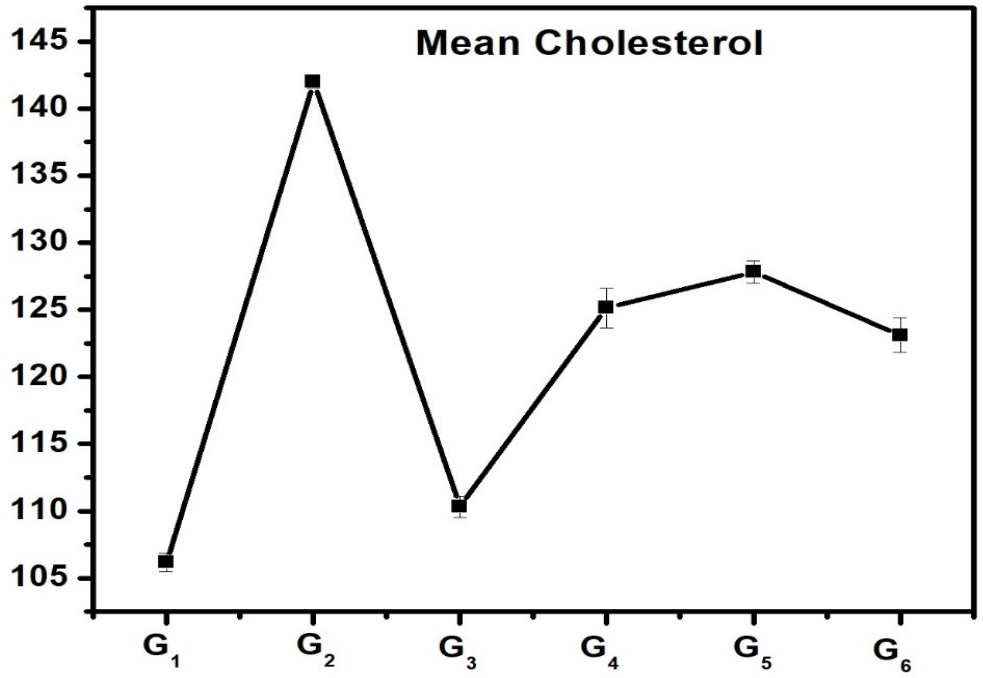


Figure 6. Mean Cholesterol in Different Treatment Groups

Triglycerides

Table 9. Total Triglycerides in Different Treatment Groups

GROUP	MEAN ± SD	F VALUE	p VALUE	SIGNIFICANCE
G_1	126.9 ± 0.91	1755.3	< 0.05	YES
G_2	209.39 ± 1.313			
G_3	157.19 ± 2.535			
G_4	171.16 ± 1.554			
G_5	176.59 ± 1.107			
G_6	170.53 ± 1.477			

The mean and standard deviations of the six groups are shown in the table. For comparing these groups, we use One-way Anova test. At 5% level of significance, the test has shown great significant difference, as the p-value obtained is less than 0.05. The mean values are signified graphically using a line graph. From this, it is clear that the mean TG of all the treatment control groups are higher than that of the standard drug, but still lower than that of the tumor bearing mice. Hence the test drugs are also effective in lowering the triglyceride level.

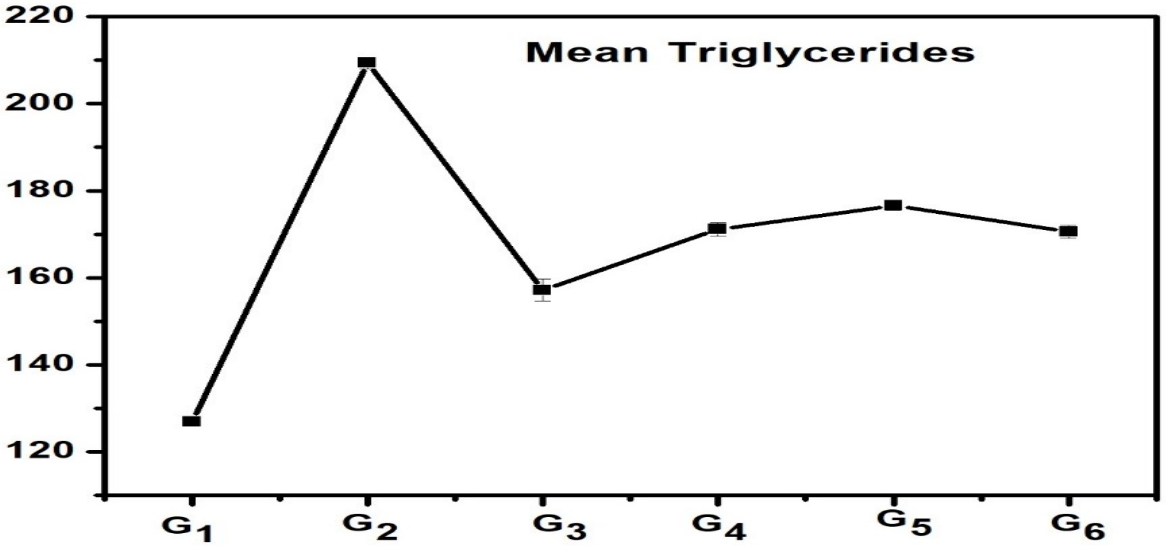


Figure 7. Mean Triglycerides in Different Treatment Groups

Aspartate Amino Transferase

Table 10. AST Levels in Different Treatment Groups

GROUP	MEAN ± SD	F VALUE	p VALUE	SIGNIFICANCE
G_1	42.603 ± 2.209	476.64	< 0.05	YES
G_2	91.197 ± 1.37			
G_3	57.212 ± 1.628			
G_4	76.4 ± 1.372			
G_5	78.6993 ± 1.784			
G_6	72.032 ± 2.843			

The mean and standard deviations of the six groups are shown in the table. For comparing these groups, we use One- way Anova test. At 5% level of significance, the test has shown great significantdifference, as the p-value obtained is less than 0.05. The mean values are signified graphically using a line graph. From this, it is clear that the mean AST of all the treatment control groups are higher than that of the standard drug and lower than that of the tumor bearing mice. Hence the drug is effective in lowering the AST levels but not as effective as the standard drug.

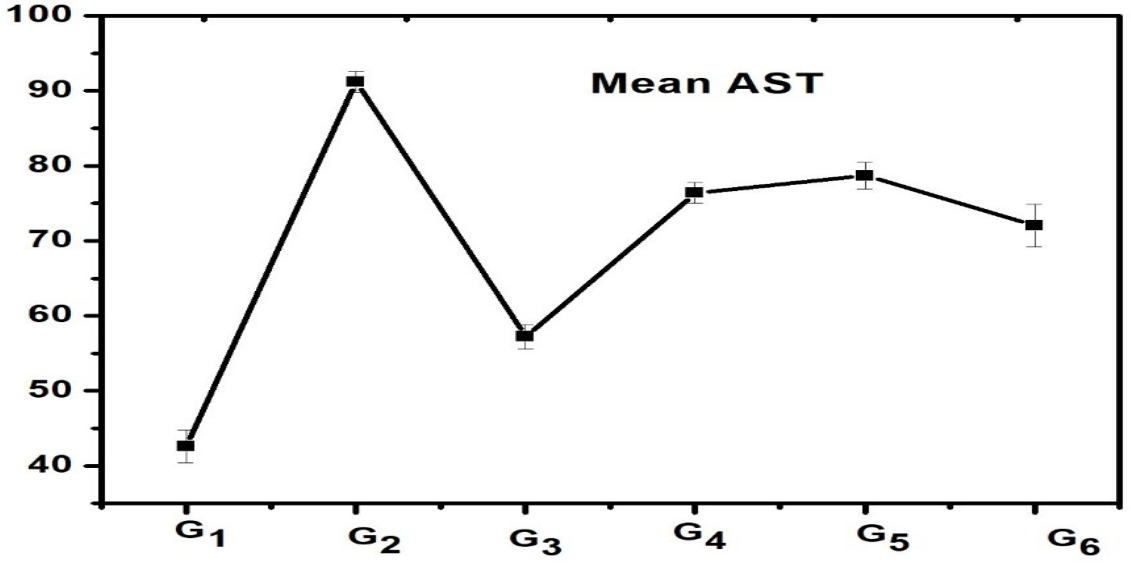


Figure 8. Mean AST in Different Treatment Groups

Alanine Amino Transferase

Table 11. ALT Levels in Different Groups

GROUP	MEAN ± SD	F VALUE	p VALUE	SIGNIFICANCE
G_1	35.69 ± 2.356	122.773	< 0.05	YES
G_2	60.272 ± 3.403			
G_3	43.6 ± 1.515			
G_4	56.113 ± 1.811			
G_5	59.87 ± 2.032			
G_6	44.3 ± 1.897			

The mean and standard deviations of the six groups are shown in the table. For comparing these groups, we use One-way Anova test. At 5% level of significance, the test has shown great significantdifference, as the p-value obtained is less than 0.05. The mean values are signified graphically using a line graph.

From this, it is clear that the mean ALT of all the treatment control groups are higher than that of the standard drug. At higher concentration the drug is as effective as the standard drug. Hence the drug is effective at higher doses for lowering the ALT levels.

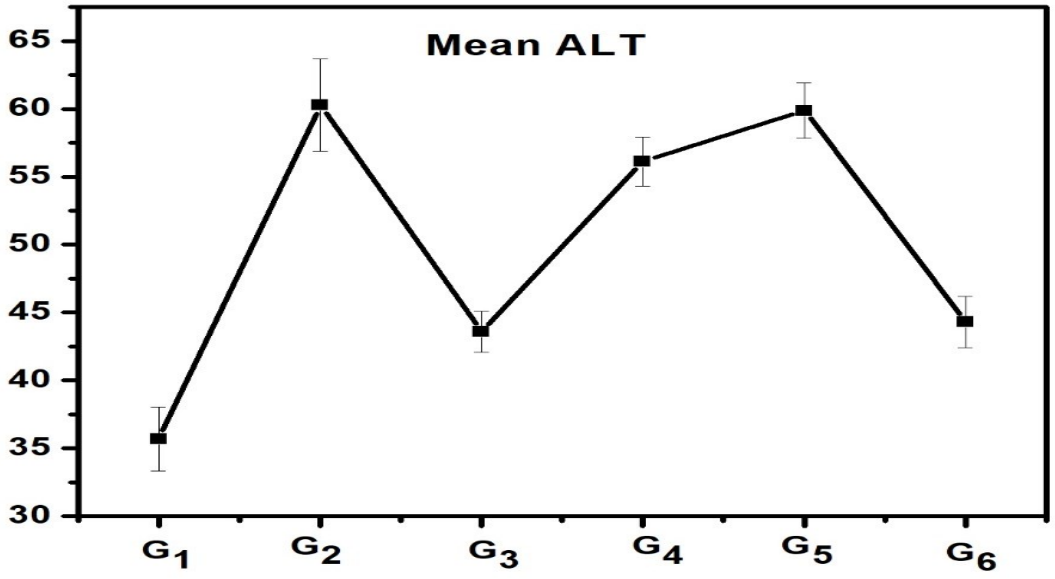


Figure 9. Mean ALT in Different Groups

Alkaline Phosphatase

Table 12. ALP Levels in Different Treatment Groups

GROUP	MEAN ± SD	F VALUE	p VALUE	SIGNIFICANCE
G_1	129.68 ± 2.471	986.058	< 0.05	YES
G_2	241.66 ± 0.907			
G_3	166.46 ± 4.483			
G_4	207.17 ± 2.991			
G_5	216.30 ± 2.24			
G_6	194.56 ± 4.019			

The mean and standard deviations of the six groups are tabulated in the table 4.36. For comparing these groups, we use One-way Anova test. At 5% level of significance, the test has shown great significantdifference, as the p-value obtained is less than 0.05. The mean values are signified graphically using a line graph. From this, it is clear that the mean ALP of all the treatment control groups are higher than that of the standard drug and at a higher concentration the test drug is effective more or less similar to the standard drug.

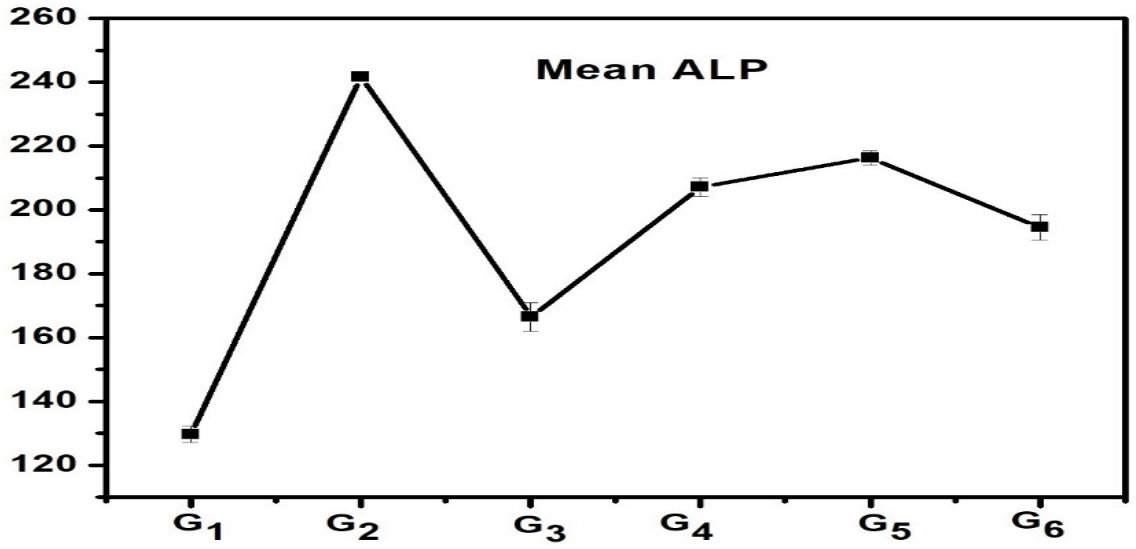


Figure 10. Mean ALP in Different Treatment Groups

The comparative effect of the test drug on liver function and lipid profile in cancer bearing mice is shown in table 4.37. In DLA-inoculated mice, there was a significant increase (P <0.001) in the levels of total cholesterol, triglycerides, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase compared to the normal group. Treatment with the test drug at doses of 200, 100 and 400 mg/Kg body weight corrected these abnormalities, bringing the levels closer to normal. The standard treatment, 5- FU produced similar results.

Table 13. Effect of test drug on serum enzymes and lipid proteins

Treatment	Cholesterol (mg/dl)	TG (mg /dl)	AST (U/L)	*ALT (U/L)*	ALP (U/L)
G1	106.18 ± 1.75	126.90±3.65	42.60 ±1.72	35.69 ±1.67	129.68 ±1.97
G2	142.18±2.52^{a**}	209.40±3.13^{a**}	91.20±1.50^{a**}	60.28±2.15^{a**}	241.66±1.72^{a**}
G3	110.32±2.58^{b**}	157.19±2.25^{b**}	57.21 ±1.91^{b**}	43.60±1.64^{b**}	166.47±1.71^{b**}
G4	125.14±1.63^{b*}	171.16±1.81^{b*}	76.40 ±1.71^{b*}	56.11±1.08^{b*}	207.17±2.10^{b*}
G5	127.82±1.23^{b*}	176.58±1.20^{b*}	78.69±1.28^{b*}	59.87±1.48^{b*}	216.30±1.78^{b*}
G6	123.10±1.10^{b**}	170.53±1.78^{b**}	72.±1.21^{b**}	44.30 ±1.30^{b**}	194.56±2.37^{b**}

All values are expressed as mean ± SEM for 6 animals in each group.
**a – Values are significantly different from the control group (G_1) at $p < 0.001$
*b – Values are significantly different from the cancer control group (G_2) at $p < 0.01$
**b – Values are significantly different from the cancer control group (G_2) at $p < 0.001$

Table 14. Effect of test drug on derived parameters

Treatment	% ILS Life span	Increase in Body weight (grams)	Cancer cell count mL × 10^6 Cells/mL	PCV %
G1	>35 days	1.89±0.49	-	13.20±2.20
G2	54%	6.39±1.85^{a**}	2.35±0.25^{a**}	30.43±3.45^{a**}
G3	90%	2.57±0.39^{b**}	1.20±0.83^{b**}	20.75±1.58^{b**}
G4	74%	5.32±1.89^{b*}	1.60±0.34^{b*}	24.81±2.82^{b*}
G5	69%	5.98 ± 1.79^{b*}	1.87±0.65^{b*}	26.35±1.89^{b*}
G6	77%	6.15±0.72^{b**}	1.44±0.41^{b**}	22.75±1.87^{b**}

All values are expressed as mean ± SEM for 6 animals in each group.

**a – Values are significantly diverse from the control group (G_1) at $p < 0.001$
*b – Values are significantly diverse from the cancer control group (G_2) at $p < 0.01$
**b – Values are significantly diverse from the cancer control group (G_2) at $p < 0.001$

All the results indicated that SK possess anticancer property though not at par with the normal drug. However, at a higher dosage, the life span was about 77% designating its potential to be used as an anticancer drug. Preparation of anticancer drug formulations with SK can promise an effective treatment.

In-vitro cytotoxic activity of *SK* at the concentrations, 6.25, 12.5, 25, 50, 100 µg/mL against SKMEL cancer cell lines was studied by MTT assay. Interestingly, a dose dependent reduction in cell viability was observed and the IC_{50} value was calculated to be **84.36 µg/mL**. The effect on PGI with the IC_{50} value 84.36 µg/mL proved that SK possess excellent antiproliferative effect in SKMEL cell lines. *In-vivo* studies done in DLA induced tumor bearing mice showed that SK at higher dose of about 400 mg/Kg was as comparable to the standard drug 5-FU. The dosages were fixed based on the acute toxicity studies. Maximum tolerated dose (MTD) was found to be 2000 mg/Kg of test drug and the $1/10^{th}$ and $1/5^{th}$ of MTD was fixed as the test drug dosage. Thus the effective doses were fixed as 200 and 400 mg/Kg for further pharmacological studies.

The antitumor effects of Snuhi *Paneeya Kshara* were demonstrated by a significant reduction in body weight gain ($p < 0.01$, $p < 0.001$) in animals treated with doses of 200, 100 and 400 mg/Kg compared to DLA tumour bearing mice. There was also a notable decrease ($p < 0.001$) in packed cell volume and viable tumour cell count with these doses of the test drug when compared to the DLA tumour control group. In terms of haematological parameters, the test drug significantly restored RBC count, haemoglobin (Hb) content, platelets count to normal levels in the cancer-bearing mice, which had been significantly decreased ($p < 0.001$) in cancer control group. Additionally, the elevated WBC count observed in the DLA control group was normalized by treatment with the test drug at 200, 100 and 400 mg/Kg doses. Despite these positive effects, the standard treatment, 5-FU, at a dose of 20 mg/Kg body weight, showed superior results across all these parameters.

The test drug also demonstrated its antiproliferative potential by affecting the liver and lipid profile in cancer-bearing mice. In DLA-inoculated mice, there was a significant increase ($p < 0.001$) in total cholesterol, triglycerides, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase levels compared to the normal group. Treatment with the test drug at doses of 200, 100 and 400 mg/Kg reversed these changes, bringing the levels closer to normal. The standard treatment, 5-FU, produced similar results. Unfortunately, histopathological comparisons between the treated groups failed to demonstrate any characteristic differences.

Ayurvedic Pharmacology explains drug action on the basis of *Rasa, Guna, Virya, Vipaka* and *Prabhava*. These parameters were advanced from the *panchamahabhootha sidhantha* which has been considered as the fundamental principle of Ayurveda. Accurate analysis of these factors stretches explanation to *karma* of any *dravya*. Modern pharmacological researchers depend upon chemical constituents of a plant for finding its probable action and implement in research studies. Detailed physico-chemical analysis will be done prior to clinical or experimental pharmacology study in any research. Considering Ayurvedic principles and fundamentals, this physico-chemical evaluation helps to substantiate its karma based on Rasa panchakas.

Kshara prepared from the Snuhi plant (*Euphorbia neriifolia*) is considered for our present pharmacology study. The whole plant was burnt into *kshara* and was studied for their phytoconstituents and pharmacological action. The drug is under constant research and discoveries for its innumerable uses and its applications especially in medical field. Ayurvedic literatures has explained the properties of *kshara* to be useful for different applications. The Snuhi plant, especially *ksheera* has been widely used for various medical practices, for eg. Bhagandara. In the same way, *kshara* prepared from Snuhi has also been a promising candidate for Ayurvedic uses. However, *SK* is least explored for its anticancer property which can provide new therapeutic implications. Preliminary Phytochemical and pharmacological analysis done through this study and previous research findings gives ideas of probable chemical activity which is considered for discussion to explore its probable mode of action.

Consequently, *Kshara* demonstrated to have the anticancer potential at a dosage of 400 mg/Kg comparable to the standard drug, 5-FU, as evident from the treatment done in DLA induced tumour bearing mice. Remarkably, *kshara* has been reported in Ayurvedic literatures as a correcting factor for cellular level metabolism due to the properties such as Tikshna (penetrating), Ushna (hot), Deepana, and Pachana (improves appetite and digestion). The reports claim that *kshara* is capable of performing the action of incision, puncture, and scarification to reduce pain. This property makes *kshara* superior over the surgical and para-surgical measures. The *kshara* can exert Tridosha derangements evenly upon the local administration. According to Sushruta *kshara* is Tridoshaghna and calm all the humors owing to its Saumyata, Pachana, Katuka, Ushna, Tikshna, Vilayana, Shodhana, Ropana, Stambhana and Lekhana properties. Saumyata refers to its burning characteristics and Pachana is the capacity to promote digestion. Katuka refers to its bitter taste, Ushna to its ability to generate heat, Tikshna to its ability to irritate, Vilayana, to its ability to cause liquefaction, Shodhana, to its ability to purify, Shoshana to its absorption promotion, Ropana to its granulation or healing, Stambhana, to its stopping or arresting nature and Lekhana-to its scraping property. The extreme hygroscopic and caustic effect can increase *kleda* and will prevent cellular metabolic processes. The cytotoxic contents of the cytoplasm and the change in pH of the cytoplasm results in the death of neoplastic cells. The Antimetabolic and Anitmitotic effect finally thus resticts the neoplasm.

In the case of SK, it can be concluded that the bioactive compounds, especially the phenolic compounds work synergistically in inhibiting proliferation of cancer cells and suggests that they may have potential for use as herbal medicine in cancer therapy. SK is reported to be possessing *katu, tikta, tikshna, vanhi deepana, ushna, Lekhana, pachana, darana, shodhana, Kapha-Vataghna* and *Tridoshaghna guna* thus attributing to the anticancer activity [10]. The wide ranges of the secondary metabolite contents and antioxidant activities of the *kshara* could be contributing to the antiproliferative effect. The presence of phenolic compounds will be useful for the standardization of SK for further pharmaceutical productions. More information on other bioactive component of SK would help us to establish a greater degree of accuracy and in the further usage of *kshara* in combination therapy.

CONCLUSION

SK samples at different concentrations have shown dose dependent inhibition of SKMEL cancer cells in the MTT assay with an IC_{50} value of 84.36 µg/mL. Henceforth, SK sample was tested for its efficacy in DLA induced tumour in mice. The life extent of the tumour induced mice increased to 74%, 69% and 77% respectively with the concentration of 200, 100 and 400 mg/Kg. In the DLA tumour bearing mice, the average life span was recorded to be 54%. These values were significant ($p < 0.001$) when compared with cancer control group mice. In 5-FU treated group, the average life span was determined as 90%, signifying its potent anticancer property. The antitumor nature of SK was further evidenced by the significant reduction ($p < 0.01$, $p < 0.001$) of body weight gain in mice treated with test drug at the dose of 200, 100 and 400 mg/Kg test drug when compared to DLA tumour control group. A significant reduction ($p < 0.001$) was also observed in packed cell volume and viable tumour cell count with the test drug dosage of 200, 100 and 400 mg/Kg when compared to the DLA tumour control. RBC count, Hb content, Platelets count were significantly decreased ($p < 0.001$) in cancer control group and were brought back to normal after treatment with 200, 100 and 400 mg/Kg of SK. WBC count was significantly increased ($p < 0.001$) in the DLA control group and was normalized by the SK treatment at the dose of 200, 100 and 400 mg/Kg. However, the standard 5-FU at the dose of 20 mg/Kg body weight produced superior results in all these parameters. DLA induced mice demonstrated significant increase

($p<0.001$) of the level of total cholesterol, triglycerides, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase when compared to the normal group. The treatment with test drug, SK at the dose of 200, 100 and 400 mg/Kg body weight reversed these changes towards the normal level. The results obtained for the dosage of 400 mg/Kg is almost comparable with that of the standard drug 5-fluorouracil.

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