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Appraisal of the Anticancer Activity of Various Formulations of *Triphala* Against Ehrlich Ascites Carcinoma In Mice

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ABSTRACT

The current investigation was delineated to find out the anticancer activity of three different formulations of *Triphala* and to find the best *Triphala* formulation for anticancer activity among the three. Aqueous extracts of *Terminalia chebula*, *Termialia bellerica* and *Phyllanthus emblica* were obtained from *Amruta* herbals, *Indore* was dissolved in purified water to make Triphala 1:1:1, 1:2:3, 1:2:4 (F1, F2, F3 respectively) formulation. The number of $1x10^6$ Ehrlich ascites carcinoma (EAC) cell was inoculated in mice. Group I served as untreated negative control while group II was considered as positive control. Group III cancerous animals received standard drug cyclophosphamide 25 mg/kg b.w., whereas group IV to VI cancerous animals were treated with F1 150, 300 and 600mg/kg b.w., group VIII to IX with F2 150, 300 and 600 mg/kg b.w and group X to XII with F3 150, 300 and 600mg/kg b.w., respectively. Different parameter such as change in body weight, determination of survival time, total ascites fluid volume, packed cell volume and haematological parameters were perceived. At the end of investigation, 6 animals from each group were immolated, blood samples were collected and WBC, RBC, Hb content was reckoned. The effect of all three formulations on reduction in body weight, tumor volume, packed cell volume, percentage increase in life span (%ILS) and haematological parameters were compared. The data revealed were suggested that all the three *Triphala* formulation possessed significant anticancer activity and *Triphala* 1:2:3 formulation at 600 mg/kg b. w., possessed maximum anticancer activity among the 9 groups compared.

Key words: *Triphala*, Antitumor activity; Ehrlich ascites carcinoma.

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INTRODUCTION

Chemotherapy is the conventional remedy for advanced or metastatic disease and anticancer activity of drugs depends on the mechanism of action of how these agents are efficacious in killing the tumor cells or in intercepting the growth of tumor cells. Nevertheless, it's efficacy can be restrained by a several factors including drug resistance, normal tissue injury, raised toxicity and ascend side effects.[1] Over years, scientists have concentrated on the antitumor activity of plants.[2] Therapeutics plants have been known to be superior sources of beneficial antitumor drugs such as epipodophyllotoxin, camptothecin, taxanes and vinca alkaloids. In spite of the invention of new drugs, cancer persists to produce the second largest cause of death in the world and assert over 6 million lives each year.[3] Consequently, the demand for research regarding new drugs that could perpetuate the life span of patients. Scientists have newly concentrated on the use of Ehrlich ascites carcinoma cells in the survey of plants revealed to heal cancer locally.[4] Ehrlich ascites carcinoma cells are transplantable tumor applied in the evaluation of anticancer drugs in mice.[5]*Triphala* means (in Sanskrit tri = three and phala = fruits) which is one of the most crucial ayurvedic medicinal preparation. It is composed of the three myrobalans, *Terminalia bellirica* (*Bibhitaki*), *Terminalia chebula* (*Haritaki*) and *Phyllanthus emblica* (*Amalaki* or the Indian gooseberry).[6]*Triphala* is herbal formulation[7] which has potential an antioxidant activity[7,8] and contains various worthwhile properties that diversity from gastroprotection to immune activity. In ayurvedic medicine, *Triphala* is applied for cleansing of colon, constipation, gastrointestinal tract, gastric disorders and colon tonifier.[1]The *Triphala* predominantly composes the equal proportions of the dried pericarps of these myrobalans.[9] Natheless, modified formulations of *Triphala* are ubiquitous and the two most prime modifications are: 1) *Triphala is* made up of one part of *Terminalia chebula*, two parts of *Terminalia bellerica* and three parts of *Emblica officinalis* (1:2:3).[6,10] 2) *Triphala* is composed of one part of *Terminalia chebula*, two parts of *Terminalia bellerica* and four parts of *Emblica officinalis* (1:2:4).[6,10] Prior anticancer investigation have all been executed with the 1:1:1 of *Triphala* and that oral remedy of *Triphala* at 25, 50 and 100 mg/kg b.w for 14 days retarded tumor growth.[11] Recent research designed to invest for all three *Triphala* combination in the EAC tumor bearing mice.

MATERIALS AND METHOD

Herbal drugs and Preparation of the Extracts:

Aqueous extracts of *Terminalia chebula, Terminalia bellerica* and *Phyllanthus emblica* was obtained from *Amruta* herbals. The extracts was preserved in a closed container below 20^0 C.

Triphala extract in 1:1:1 ratio (F1): one part of *T. chebula*, one part of *T. bellerica* and one part of *E. officinalis* were taken and dissolved in water to produce 1:1:1 ratio. *Triphala* extract in 1:2:3 ratio (F2): one part of *T. chebula*, two part of *T. bellerica* and three part of *E. officinalis* were taken and dissolved in water to make 1:2:3 ratio. *Triphala* extract in 1:2:4 ratio (F3): one part of *T. chebula*, two parts of *T. bellerica* and four parts of *E. officinalis* were taken and dissolved in water to gain 1:2:4 ratio. All extracts were freshly prepared every day before dosing the animals.

Chemicals

All chemical, reagents, solvent used were acquired from Fine Chemicals Pvt. Ltd., Mumbai, Sigma chemical, Loba chemical, Mumbai.

Tumor cells line

Ehrlich ascites carcinoma (EAC) cell line inoculated mice was supplied by Division of Radiobiology and Toxicology, Manipal Life science Centre, Manipal University, India. The EAC cells were maintained by serial intraperitonial (i.p) transplantation in mice. Full grown tumor cell-line was aspirated from the mouse peritoneum, washed trice with 0.9% saline. The number of $1x10^6$ viable cells injected intraperitoneally into a new healthy mouse.

Animals

Healthy female adult Swiss albino mice weighing 20-25g was obtained from the Central Animal Facilities of the St. Johns Pharmacy College, Bangalore, were maintained in husbandry condition. The animals were kept in the hygienic cages during the investigation period. The animals were permitted free ingress to standard laboratory cube pellets and drinking water *ad libitum.* The research protocol was ratified by Institutional Animal Ethics Committee (IAEC), St. John's pharmacy college, Bangalore (Ref. no. IJAHSM / IAEC / 2008 / 010).

Treatment designed

Healthy, adult Swiss albino mice were divided into 12 groups consisting of 12 animals in each group. All the animals in each group, excluding group 1 received 10^6 EAC cells/mouse i.p. Group 1 and 2 was vehicle and EAC control respectively. Group 3 was administrated by standard drug Cyclophosphamaide 25 mg/kg b.w, i.p., group 4, 5 and 6 were administered, orally with formulation of F1- (Triphala 1:1:1), 150,300 and 600 mg/kg b.w, respectively, group 7, 8 and 9 were administered, orally with formulation of F2- (Triphala 1:2:3), 150,300 and 600 mg/kg b.w, respectively and group 10, 11 and 12 were administered, orally with formulation of F3- (Triphala 1:2:4), 150,300 and 600 mg/kg b.w, respectively. Treatments were done the 6 hours after the inoculation of EAC for 9 days at 24 hours interval.

Tumor growth response:

The anticancer effect of plant extracts were appraised by change in packed cell volume, tumor volume, median survival time (MST) and percentage increase in life span (%ILS). The tumor volume was estimated by taking the ascetic fluid in a graduated centrifuge tube after its collection from the peritoneal cavity; the total volume of fluid was centrifuged at 2000 rpm for 20 minutes for ascertaining the packed cell volume. MST and %ILS were verified.[7]

Hematological parameters

At the end of investigation, on day 10, blood was collected from retro-orbital of mice for reckoning of white blood cell (WBC) count, red blood cells (RBC) count and the hemoglobin (Hb) content by standard procedures.

Statistical analysis

The data were manifested as mean ± S.E.M (n=6). The results were statistically analyzed by means analysis of variance (ANOVA) followed by Dunnett's post hoc test where the difference was contemplated significant if $p < 0.05$.

RESULTS AND DISCUSSION

Preclinical researches revealed that *Triphala* was advocated to be used as chemoprotective,[10,12] radioprotective.[7,8,13] in the past one decade and also recommended the cytotoxic effects of *Triphala* were negligible or less in the normal cells like human pancreatic ductal epithelial cells.[11] The guideline for considering the value of anticancer drug is the increase of life span of animals and decline the leukemic cells .[10] The present investigation indicated that *Triphala* 1:2:3 at 600 mg/kg administered to EAC mice significantly ascended the life span than that of the EAC control and then all other doses and formulation. As compared to the EAC control group, *Triphala* 1 :2 :3 600mg/kg treated group II has shown significant($p < 0.05$) increase in the life span than the rest of the groups[Table 1]. In addition, the reduction of tumor volume and arose survival time of mice suggest the delaying impact of *Triphala* on cell division.[10,11] All the three Triphala formulations exhibited the significant decline in body weight ($p < 0.001$), tumor volume ($p < 0.001$) and packed cell volume ($p < 0.001$) when compared with the EAC control group. Ascetic tumor reduction surveys on the prophylactic remedy of three aqueous extracts of Triphala exhibited notable tumor reducing property and the aqueous extract of *Triphala* 1: 2: 3 ratio at 600 mg/kg evincing better tumor reduction property [Table 2]. Treatment with the all three *Triphala* combination at low, medium and high dose significantly increased the RBC level and Hb content when compared to the EAC control ($p < 0.001$)[Table 3]. *Triphala* 1:2:3 at 600 mg/kg displayed better effect than other combinations as compared with cyclophosphamide ($p < 0.01$) and EAC

control ($p < 0.001$). The WBC count has been decreased significantly when compared with the EAC bearing mice and restored more towards the normal level. *Triphala* 1:2:3 at 600 mg/kg showed better activity when compared to the rest of the formulation being evaluated, when compared to the cyclophosphamide ($p < 0.05$). In the cancer chemotherapy the crucial problems are myelosuppression and anemia.[11]The anemia encountered in tumor bearing mice may be due to either iron deficiency or hemolytic or myelopathic condition.[14] The restoration of hematological parameter as a result of treatment with the *Triphala* indicates the protective action on hematopoietic system. The previous phytochemical evaluation of *Triphala* proclaimed the presence of the phenolic compound such as luteolin, ellagic acid, gallic acid and tannic acid which have a chemo preventive role through their antioxidant effects and angiogenesis.[15,16]

Table 1: Effect of *Triphala* on mean survival time in EAC tumor bearing mice

Groups	Treatment	Dose	Mean survival time	ILS %
I	Normal	-	-	-
II	EAC control + solvent	20 ml/kg	14.67±1.41	
III	Cyclophosphamide	25 mg/kg	23.67±2.09 ^{r}	61.35
IV		150 mg/kg	19.17±0.70	30.68
V	*Triphala* 1:1:1	300 mg/kg	19.67±1.31	34.08
VI		600 mg/kg	18.33±1.20	24.49
VII		150 mg/kg	18.67±1.36 ^{x}	27.27
VIII	*Triphala* 1:2:3	300 mg/kg	17.67±1.56	20.45
IX		600 mg/kg	21±1.86 ^{p}	43.15
X		150 mg/kg	17.5±0.62 ^{y}	19.29
XI	*Triphala* 1:2:4	300 mg/kg	17.5±0.67	19.29
XII		600 mg/kg	15±0.63 ^{z}	2.25

Values are mean ± S.E.M. (n=6).p values:, $p < 0.05$, $r < 0.001$ as compared with EAC control + solvent. $x < 0.05$, $y < 0.01$, $z < 0.001$, as compared to cyclophosphamide (by one way ANOVA followed by Dunnett's multiple comparison test) Whereas, groups I =normal animal, II = EAC induced + vehicle treatment, III = EAC induced + Cyclophosphamide 25 mg/kg. IV = EAC + Triphala 1:1:1 150 mg/kg, V = EAC + Triphala 1:1:1 300 mg/kg, VI = EAC + Triphala 1:1:1 600 mg/kg, VII = EAC + Triphala 1:2:3 150 mg/kg, VIII = EAC + Triphala 1:2:3 300 mg/kg, IX = EAC + Triphala 1:2:3 600 mg/kg, X = EAC + Triphala 1:2:4 150 mg/kg, XI = EAC + Triphala 1:2:4 300 mg/kg, XII = EAC + Triphala 1:2:4 600 mg/kg.

Table 2: Effect of Triphala on body weight analysis and tumor growth response against EAC induced animals.

Groups	Increase in body weight (gms)	Tumor volume (mL)	Packed cell volume (mL)	% decrease in body weight
I	0.64 ± 0.26	-	-	-
II	8.95 ± 0.47 ^{c}	8.18 ± 0.26	4.8 ± 0.30	
III	1.85 ± 0.56 ^{r}	1.2 ± 0.11^{r}	0.55 ± 0.20^{r}	79.33
IV	4.15 ± 0.37^{c,r,y}	4.63 ± 0.46^{r,z}	2.3 ± 0.22^{r,z}	53.63
V	4.56 ± 0.54^{c,r,z}	4.55 ± 0.25^{r,z}	2.13 ± 0.13^{r,z}	49.05
VI	5.03 ± 0.19^{c,r,z}	4.675 ± 0.25^{r,z}	2.48 ± 0.15$^{r,\ z}$	43.80
VII	4.93 ± 0.10^{c,r,z}	4.93 ± 0.31^{r,z}	2.55 ± 0.13^{r,z}	44.92
VIII	4.78 ± 0.19^{c,r,z}	4.88 ± 0.26^{r,z}	2.48 ± 0.05^{r,z}	46.59
IX	3.85 ± 0.49^{c,r,x}	3.85 ± 0.37^{r,z}	1.88 ± 0.08^{r,z}	56.98
X	5.97 ± 0.81^{c,r,z}	5.78 ± 0.23^{r,z}	2.8 ± 0.23^{r,z}	33.30
XI	6.18 ± 0.10^{c,r,z}	6.15 ± 0.10^{r,z}	3.13 ± 0.40^{r,z}	30.95
XII	6.07 ± 0.34^{c,r,z}	5.95 ± 0.37^{r,z}	2.95 ± 0.16^{r,z}	32.18

Values are mean ± S.E.M. (n=6).p values: a < 0.05, b< 0.01, c< 0.001, as compared with normal control. P < 0.05, q< 0.01, r < 0.001, as compared with EAC control + solvent. x < 0.05, y< 0.01, z < 0.001 , as compared to cyclophosphamide (by one way ANOVA followed by Dunnett's multiple comparison test)Whereas, groups I =normal animal, II = EAC induced + vehicle treatment, III = EAC induced + Cyclophosphamide 25 mg/kg. IV = EAC + Triphala 1:1:1 150 mg/kg, V = EAC + Triphala 1:1:1 300 mg/kg, VI = EAC + Triphala 1:1:1 600 mg/kg, VII = EAC + Triphala 1:2:3 150 mg/kg, VIII = EAC + Triphala 1:2:3 300 mg/kg, IX = EAC + Triphala 1:2:3 600 mg/kg, X = EAC + Triphala 1:2:4 150 mg/kg, XI = EAC + Triphala 1:2:4 300 mg/kg, XII = EAC + Triphala 1:2:4 600 mg/kg.

Table 3: Effect of Triphala on hematological parameters on 14th day in normal and EAC tumor bearing mice.

Groups	Treatment	Dose	WBC(x10^{6}/ml)	RBC(x10^{9}/ml)	Hb(gm%)
I	Normal	-	7.24±0.23	5.67±0.24	12.9±0.44
II	EAC control	20 ml/kg	21.78±1.31^{c}	3.55±0.04^{c}	8.1±0.28^{c}
III	Cyclophosphamide	25 mg/kg	10.71±0.27$^{c,\ r}$	5.26±0.09^{a,r}	12.08±0.39^{r}
IV	1:1:1	150 mg/kg	14.65±0.17$^{c,r,\ z}$	4.82±0.04^{c,r,z}	9.71±0.21^{c,y}
V		300 mg/kg	13.21±0.15^{c,r,z}	4.95±0.10^{c,r,y}	11.13±0.39^{a,r}
VI		600 mg/kg	13.26±0.42^{c,r,z}	4.77±0.02^{c,r,z}	10.67±0.26^{b,r}
VII	1:2:3	150 mg/kg	15.21±0.18^{c,r,z}	4.73±0.04^{c,r,z}	8.92±0.08^{c,z}
VIII		300 mg/kg	14.15±0.16^{c,r,z}	4.82±0.06^{c,r,z}	9.59±0.58^{c,y}
IX		600 mg/kg	12.13±0.44^{c,r,x}	4.97±0.03^{c,r,y}	11.54±0.23^{r}
X	1:2:4	150 mg/kg	16.91±0.15^{c,r,z}	4.59±0.01^{c,r,z}	8.32±0.61^{c,z}
XI		300 mg/kg	14.92±0.41^{c,r,z}	4.27±0.04^{c,r,z}	10.55±0.47
XII		600 mg/kg	16.18±0.54^{c,r,z}	4.40±0.05^{c,r,z}	9.29±0.61 ^{c}

Values are mean ± S.E.M. (n=6).p values: a < 0.05, b< 0.01, c< 0.001, as compared with normal control. P < 0.05, q< 0.01, r < 0.001, as compared with EAC control + solvent. x < 0.05, y< 0.01, z < 0.001 , as compared to cyclophosphamide (by one way ANOVA followed by Dunnett's multiple comparison test)Whereas, groups I =normal animal, II = EAC induced + vehicle treatment, III = EAC induced + Cyclophosphamide 25 mg/kg. IV = EAC + Triphala 1:1:1 150 mg/kg, V = EAC + Triphala 1:1:1 300 mg/kg, VI = EAC + Triphala 1:1:1 600 mg/kg, VII = EAC + Triphala 1:2:3 150 mg/kg, VIII = EAC + Triphala 1:2:3 300 mg/kg, IX = EAC + Triphala 1:2:3 600 mg/kg, X = EAC + Triphala 1:2:4 150 mg/kg, XI = EAC + Triphala 1:2:4 300 mg/kg, XII = EAC + Triphala 1:2:4 600 mg/kg.

CONCLUSION

All three *Triphala* formulation possessed anti-cancer activity in EAC cell inoculated Swiss albino mice, with Triphala 1:2:3 formulation at 600 mg/kg b.w dose has exhibited significant increase of lifespan, diminished in tumor volume, improvement in the hematological parameters when compared to the rest of groups. Inevitably, *Triphala* 1:2:3 ratio formulation has superior anticancer activity than the typical 1:1:1 ratio and 1:2:4 ratio formulation.

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