ISSN 2582 – 6050

Available Online at www.ijpba.info
International Journal of Pharmaceutical & Biological Archives 2023; 14(3):123-132

RESEARCH ARTICLE

Evaluation of Anti-tumor Properties of Herbal Formulations against Ehrlich Ascites Solid Tumor Mice Model

Ashok Kumar Gupta[1], Manish Kumar[1]*, Javed Ali[2], Arti Gupta[3]

[1]*Project Hospital Obra, Uttar Pradesh Rajya Vidyut Utpadan Nigam Limited, Lucknow, Uttar Pradesh, India,*
[2]*Department of Pharmacology, Aligarh Muslim University, Aligarh, Uttar Pradesh, India,*
[3]*Department of SNCU, 100 Bedded Joint District Hospital, Kasganj, Uttar Pradesh, India*

Received: 25 May 2023; Revised: 12 June 2023; Accepted: 25 July 2023

ABSTRACT

Saraca indica (Ashoka) is an important plant belonging to the family *Fabaceae*. It essentially contains glycosides, tannin, saponin, flavonoids, and sterol. It possesses a variety of activities such as analgesic, antipyretic, fungitoxic, anthelmintic, antidiabetic, larvicidal activity, antimicrobial activity, central nervous system depressant activity, antiulcer activity, anti-cancer, and anti-inflammatory activity. The anticancer principle from *S. indica* flowers indicated 50 percent cytotoxicity (*in vitro*) in Dalton's lymphoma ascites and Sarcoma-180 tumor cells at a concentration of 38 mug and 54 mug, respectively, with no activity against normal lymphocytes but preferential activity for lymphocytes derived from leukemia patients. A new plant is also taken as they have no reported activity for *in vivo* in cancer. One such plant is *Solanum xanthocarpum* Schrad. and Wendl. (Family: *Solanaceae*). Various therapeutic properties are attributed to it, particularly in the cure of asthma, chronic cough, and catarrhal fever. Plant contains alkaloids, sterols, saponins, flavonoids, and their glycosides and also carbohydrates, fatty acids, and amino acids. The exact mechanism of action of Kantkari is still unknown due to not reported yet, so the present study was designed to investigate the anti-tumor potential of herbal drugs (Ashoka and Kantkari) against Ehrlich ascites solid tumor mice model. Solid tumors were induced by injecting Ehrlich ascites carcinoma (EAC) cells (15×10^6/mice) subcutaneously in right hind limb. Treatment with drugs (Ashoka and Kantkari) showed significant decrease in body weight, solid tumor volume, and tumor weight in EAC tumor-bearing mice at a dose of 400 mg/kg and 200 mg/kg, respectively. Ashoka increased the mean survival time of tumor-bearing mice and percentage increase in life span (was found to be 90.53% and Kantkari is 88.33%, respectively, which is lower than the standard drug Vincristine but Kantkari shows that the tumor inhibition is more significant than Ashoka. Results of with hematoxylin and eosin and immunohistochemistry explained the possible mechanism of action of drugs (Ashoka and Kantkari). Decreased expression of vascular endothelial growth factor indicated the anti-angiogenic property. Immunochemical analysis of solid tumor showed increased expression of caspase-3 suggested that drugs (Ashoka and Kantkari) induced apoptosis in tumor cells. Findings of the present study explored the possible anti-tumor properties of drugs (Ashoka and Kantkari) and can be room for further evaluations in clinical studies.

Keywords: Apoptosis, caspase-3, Ehrlich ascites solid tumor, *Saraca indica*, *Solanum xanthocarpum*, vascular endothelial growth factor

***Corresponding Author:**
Manish Kumar,
E-mail: manishpharma20@gmail.com

INTRODUCTION

Current scenario of health-care system facing the most of the challenges related to the complete cure

of many deadly diseases in which cancer is on top of the list. It has been reported that cancer is the second leading cause of death in the world after cardiovascular diseases and it is projected that it would become primary cause of death within the coming years worldwide.[1] According to GLOBOCAN 2012, an estimated 14.1 million new cancer cases and 8.2 million cancer-related deaths occurred in 2012, compared with 12.7 million and 7.6 million, respectively, in 2008. The prevalence estimates for 2012 show that there were 32.6 million people (over the age of 15 years) alive who had had a cancer diagnosed in the previous 5 years. Projections based on the GLOBOCAN 2012 estimates predict a substantive increase to 19.3 million new cancer cases per year by 2025, due to growth and aging of the global population. More than half of all cancers (56.8%) and cancer deaths (64.9%) in 2012 occurred in less developed regions of the world, and these proportions will increase further by. According to these figures lead, identification of novel targeted molecules for chemoprevention is still a major task for the researches.[1]

Despite of extensive research during recent decades bring out some innovative outcomes with very effective anticancer agents but, current anti-tumor chemotherapy still suffers from major limitations – the first is the lack of selectivity of conventional chemotherapeutic agents for cancer tissues, bringing about unwanted side effects. The second is the acquisition by cancer cells of multiple-drug resistance. Unwanted side effects of anti-tumor drugs could be overcome with agents capable of discriminating tumor cells from normal proliferative cells and the resistance is minimized using combined modality approach with different complementary mechanism of action. Considering all the aspects of currently available therapies for chemoprevention, there is quest of a potential anti-tumor agent and plant kingdom could be the potential source of chemicals with anti-tumor and cytotoxic activities.[2] New drugs generated from secondary metabolites of plants represent an alternative source of anticancer agents, which frequently seem to be more effective and/or less toxic. Nowadays, herbals have been used for its diverse pharmacological properties as they cause less adverse effects including cytotoxic and cancer chemopreventive properties.[3]

The present study is designed to evaluate the anticancer properties of the herbal plants (Ashoka and Kantkari). We have explored that the possible outcomes which are predicted herbal plants (Ashoka and Kantkari) are a potential anticancer agent.

MATERIALS AND METHODS

Plant

Two herbal drugs, that is, Ashoka and Kantkari taken as test compounds. Vincristine (Cytocristin) has purchased from Ark Pharmaceuticals, is used as a standard drug based on previous literature survey.

The fruit sample *of Solanum xanthocarpum* Schard. And Wendl. Kantkari was collected from Delhi/NCR, Ref.No. NISCAIR/RHMD/Consult/2015/2824/17 identified and authenticated by Dr. (Mrs) Sunita Garg, Chief Scientist, Raw Material Herbarium and Museum, Delhi (RHMD) CSIR-NISCAIR.

The flower sample *of Saraca asoca*, Ashoka, was collected from Delhi/NCR, Ref.No. NISCAIR/RHMD/Consult/2015/2824/17-1 identified and authenticated by Dr. (Mrs) Sunita Garg, Chief Scientist, Raw Material Herbarium and Museum, Delhi (RHMD) CSIR-NISCAIR.

Animals

Swiss albino mice (*Mus musculus*) either sex, 5–6 weeks old, weighing 20–30 g were used for the present study. The animals were housed in standard poly carbonate cages and feed with standard pellet diet (golden feed) under standard laboratory conditions (26 ± 1°C, 12-h light: 12-h dark cycle) with food and water. The animals were procured from the institute's animal house and acclimatized to laboratory conditions for 7 days before commencement of the experiment. The protocol for this present study was approved by the Institutional Animal Ethics Committee (Protocol no.CPCSEA/AIP/2014/08/004). Health condition of animals was observed daily.

Extraction of Plant Material

The extraction process was carried out to get the clear solution, then 10.0 g of Ashoka and Kantkari, respectively, was taken and extraction was carried out in 500 mL of aqueous for 8 h using Soxhlet Apparatus until the clear solution is obtained. As the clear solution was noticed, the extraction process was stopped and it was allowed to cool. The extracts were filtered using vacuum filter and concentrated under vacuum to carry out the further process.

Instruments

1. Remi cooling centrifuge. (C-24BL) Remi Elektrotechnik limited (VASAI–INDIA)
2. Electronic balance Type AX 200. SHIMADZU CORPORATION, JAPAN
3. Electronic balance. Type ELB 300. SHIZMADZU CORPORATION, SHIMADZU PHILIPPINES MANUFACTURING INDUSTRY (SEM)
4. Micropipette (100–1000 μL), Riviera, Riviera Glass Pvt. Ltd.
5. Micropipette, Eppendorf industry, GERMANY
6. Digital Vernier Caliper (0–150 mm), AEROSPACE
7. Neubauer hemacytometer
8. Disposable syringes of 1 mL (u-40 unit) and 2, 5 mL [Table 1].

Methods

Tumor system

EAC collected from donor mice (Swiss albino) of 20–30 g body weight of either sex be suspended in sterile isotonic phosphate-buffered saline (PBS). A fixed number of viable cells will implant into the peritoneal cavity of each recipient mouse. The tumor cells multiplied relatively freely within the peritoneal cavity. The cells will with drawn by sterile disposable syringe and diluted with PBS.[4] The viability of the cells was 96.8% as judged by trypan blue exclusion assay.

Table 1: ELISA kits

Purpose	Kit	Company
VEGF estimation	Mouse vascular endothelial growth factor immunoassay	R&D system, USA
Caspase-3 estimation	Caspase-3 colorimetric assay kit	BIOVISION, US

VEGF: Vascular endothelial growth factor

Ehrlich ascites tumor (EAT) model

Mice were inoculated with EAC cells 15×10^6 (0.2 mL/mice). First day of tumor implantation was assigned as day 0. On day 1, mice were divided into five groups. Treatment of herbal formulations (oral) started from day 6 from tumor implantation and continued for 30 day compared with vincristine 0.5 mg/mL/d (i.p). Body weight changes were measured from day 3 to day 30 at an interval of every 3 day, six mice were sacrificed on day 31, and tumor was excised and tumor mass was weighed and volume of solid tumor was gauged using Vernier caliper and was calculated using formula $A \times B^2 \times 0{:}52$, where A and B are the longest and the shortest diameter of tumor, respectively. After that, tumors were preserved in 10% buffered formalin and processed for histopathological and immunohistochemical investigations.[5]

Treatment plan

All the drugs administered i.p. and oral as described in table. Doses of standard drug and herbal formulations were selected[6] and the pilot study conducted in the laboratory.

Standard drug

- *Vincristine*: 0.5 mg/kg/d (i.p.)

Herbal formulations

- *S. xanthocarpum* (Kantkari): 40 mg/kg/d (oral)
- *S. ashoka*: 80 mg/kg/d (oral).

Tumor volume

The volume of solid tumor was measured using Vernier caliper and was calculated using formula ($A \times B^2 \times 0{:}52$), where A and B are the longest and the shortest diameter of tumor, respectively.[7]

Percentage tumor volume inhibition (%TVI)

The percent tumor volume inhibition in treated groups was calculated as follows.[7]

$$\%Tumor\ inhibition = \frac{Av.tumor\ volume\ in\ control\ group - Av.tumor\ volume\ in\ test\ group}{Av.tumor\ volume\ in\ control\ group} \times 100$$

Percentage increase in life span (%ILS)

Percentage increase in life span and mean survival time was calculated using formula follows.[7]

$$Mean\ survival\ time = \sum \frac{Survival\ time\ (days)\ of\ each\ mouse\ in\ a\ group}{Total\ no.of\ mice}$$

% ILS = MST of treated group × 100⁄MST of control group.

Experimental design [Table 2]

Treatment groups and doses of test compounds are designed and performed Histological studies, Immunohistochemical analysis, vascular endothelial growth factor (VEGF) and Caspase-3 study.

Histological studies

After 30 days of drug treatment, six mice were sacrificed that tumor masses were excised and fixed in 10% formalin. The formalin fixed gels were processed using the automated tissue which involved dehydration using followed by clearing with Xylene and finally impregnated in paraffin blocks. Sections (~5 μm) were cut using rotary microtome and stained with hematoxylin and eosin. All sections were dehydrated, mounted with covers slips and views under light microscope. The slides were examined for histopathological changes such as inflammatory reaction, necrosis, mitotic figures, and apoptotic index.[7]

Table 2: Treatment groups and doses of test compound

Group no.	Groups	Dose (mg/kgbw/d)
1	EAT Control	-
2	EAT+ASHOKA	400
3	EAT+KANTKARI	200
4	EAT+VINCRISTINE	0.5

EAT: Ehrlich ascites tumor

Immunohistochemical analysis

Formalin fixed tissues were processed and embedded into paraffin. Sections ~ 5 μm thick were deparaffinized in xylene and alcohol and then rehydrated in demonized water. Immunohistochemical staining procedures were then performed.

Vascular endothelial growth factor (VEGF)

VEGF plays major role in the formation of new blood vessels in tumor mass and surrounding tissue which results in progression of cancer more rapidly. To determine the anti-angiogenic property of CAR, immunohistochemical detection of VEGF has been performed in the present study. Immunostaining of paraffin embedded sections was performed by streptavidin-conjugated horseradish peroxidase complex method using a vectastain elite ABC kit (Vector laboratory, CA, USA). ~5 μm thick sections deparaffinized and incubated in PBS for 30 min. They were then treated with 0.3% hydrogen peroxide and incubated with 10% normal goat serum to block non-specific binding. The section was then incubated with rabbit anti-human VEGF monoclonal antibody (diluted 1:500) or control rabbit serum at room temperature for 2 h. They were then washed with PBS and exposed to biotinylated anti-rabbit immunoglobulin G (IgG) followed by treatment with streptavidin-conjugated horseradish peroxidase and stained with 3,3‘-diaminobenzidine with 0.15% hydrogen peroxideand Counterstaining is carried out with Harris hematoxylin (sigma) stain. VEGF immunoreactivity was observed in cytoplasm of tumor cells. The grade immunostaining was observed at ×400 magnification, based on both the staining intensity (negative, faintly stained, or intensely stained) and number of positive cells (0%, 50% or less, more than 50%). The results of were semi-quantitatively as (−/+) when there were no positive cells, weakly positive (+) when the staining was faint or positive cells were 50% or less, moderately stained when the staining was between 50% and 70% (++) and strongly positive (+++) when the number of intensely stained cells was >70%.

Caspase-3

To check the CAR-induced apoptosis in tumor cells, immunohistochemical analysis of caspase-3 was performed. Deparaffinized sections were incubated in PBS for 30 min. They were then treated with 0.3% hydrogen peroxide and incubated with 10% normal goat serum to block nonspecific binding. The sections were then incubated with the mouse caspase-3 antibody at 1:200 dilutions or control rabbit serum at room temperature for 2 h. They were then washed with PBS and exposed to biotinylated anti-rabbit IgG, followed by treatment with streptavidin-conjugated horseradish peroxidase and stained with 3,3·-diaminobenzidine with 0.15% hydrogen peroxide and Counterstaining is carried out with Harris hematoxylin (sigma) stain. Five high-power fields in different areas on each were examined caspase-3 positive cells. The results of were semi-quantitatively as (−/+) when there were no positive cells, weakly positive (+) when the staining was faint or positive cells were 50% or less, moderately stained when the staining was between 50% and 70% (++) and strongly positive (+++) when the number of intensely stained cells was >70%.

RESULTS

Effect on Body Weight

EAT-bearing mice (group 1) showed increase in body weight from 0.17 g on day 5 to 14.25 g on day 30, whereas VINCRISTINE (0.5 mg/kg) group showed increase in mean body weight from day 5 (23.91 ± 2.81 g) on day 15 (27.33 ± 2.78 g). Interestingly on day 15, the body weight of the VINCRISTINE group animals was found to be decreased (26.75 ± 1.96), along with the total increase of 8.5 g body weight as compared to EAT control. The animals treated with Ashoka (400 mg/kg) and Kantkari (200 mg/kg) was shown body weight 0.75 g on day 5 to 7.0 g on day 30. ASHOKA (400 mg/kg) group not showed any significant increase in mean body weight from day 5 (25.08 ± 0.86) on day 20 (25.58 ± 1.53 g) as compared to EAT control, but total increase in mean body weight was found to be 5.25 g. On the other hand, KANTKARI 200 mg/kg, group exhibited significant reduction against EAT bearing mice. On day 15, body weight of KANTKARI 200 mg/kg animals treated group reduced from 28.33 ± 1.12 g to 26.66 ± 1.21 g. This was followed by a continuous reduction in mean body weight which was observed throughout the experiment. KANTKARI 200 mg/kg group animals showed a total decrease in body weight (2.83 g) at the end of experiment [Figure 1].

Effect on Solid Tumor Volume

In EAT group, average tumor volume was found to be significantly higher than the treatment groups. In EAT control, the average tumor volume was found to be 5356.20 mm^3 followed by Kantkari (200 mg/kg) group average tumor volume as 2933.92 mm^3 as well as Ashoka 400 mg/kg significantly reduced the tumor volume 2873.01 mm^3, respectively. Maximum reduction in solid tumor volume was observed with Kantkari (200 mg/kg) which was found significant as compared to disease control group. The %TVI caused by various concentrations of Ashoka and Kantkari was recorded to be 46.36%, 45.22% at a dose of 400 and 200 mg/kg/d, respectively. Tumor growth inhibition caused by vincristine was found to be 49.97% [Figure 2 and Table 3].

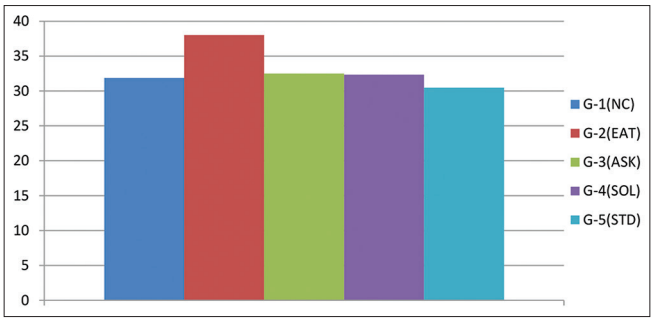

Figure 1: Shows body weight variation

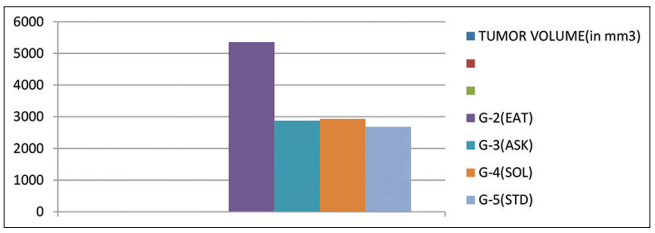

Figure 2: Solid tumor volume

Effect on Mean Survival Time

Mean survival time of EAT-bearing mice was 25.5 days, whereas treatment with Kantkari at a dose of 200 mg/kg significantly increased the MST from 24.5 days to 36.83 days as compared to EAT control. %ILS caused by standard drug vincristine at dose of 0.5 mg/kg/d was 97.76%, whereas treatment with Ashoka and Kantkari, the %ILS which is 90.53%, 88.33% at a dose of 400 and 200 mg/kg, respectively. Highest observed percent increase in life span was 97.76% with vincristine 0.5 mg/kg [Figure 3 and Table 4].

Effect on Tumor Weight

Average tumor weight of EAT-bearing mice (group 1) is 25.09 g. Treatment with vincristine (0.5 mg/kg) showed significantly decreases the tumor weight (17.74 g) as compared to EAT control. In contrast, treatment with Ashoka and Kantkari at a dose of 400 and 200 mg/kg significantly exhibit marked decrease in tumor weight 20.62 g and 19.06 g, respectively. Minimum average tumor weight by herbal treatment was found to be 19.06 g which was result of treatment with Kantkari 200 mg/kg. It would be depicted that Kantkari treatment was proven to reduce the tumor slightly burden in EAT-bearing mice [Figures 4 and 5, Table 5].

Table 3: Effect of herbal drugs on solid tumor volume against EAT-bearing mice

Groups	Solid tumor volume (mm^3)	Percentage tumor volume inhibition
EAT CONTROL	5356.20	-
EAT+ASHOKA	2873.01	46.36
EAT+SOLANUM	2933.92	45.22
EAT+VINCRISTINE	2679.64	49.97

EAT: Ehrlich ascites tumor

Table 4: Effect of Ashoka and Kantkari on mean survival time against EAT-bearing mice

Groups	Mean survival time (days)	Percent increase in life span
EAT CONTROL	25.5	-
ASHOKA	27.16	90.53
SOLANUM	26.5	88.33
VINCRISTINE	29.33	97.76

EAT: Ehrlich ascites tumor

Table 5: Effect of Ashoka and Kantkari on tumor weight against EAT-bearing mice

Groups	Tumor weight (g)
EAT CONTROL	25.09
ASHOKA	20.62
SOLANUM	19.06
VINCRISTINE	17.74

EAT: Ehrlich ascites tumor

Histological studies

As shown in Figure 6, EAT group showed cods of malignant cells, necrosis deep inside the tumor margins, and tumor infiltration. Vincristine and Kantkari 200 checked the size of tumor in a similar manner. Kantkari 200 reduced the tumor and inflammatory infiltration and also decreased number of mitotic figures were found in high-power field as compared to EAT control. Kantkari-induced apoptosis can be explained by higher number of apoptotic bodies [Figure 6 and Table 6].

Immunohistochemical Analysis

Caspase-3 plays important role in programmed cell death. In EAT control, no positive cells were found.

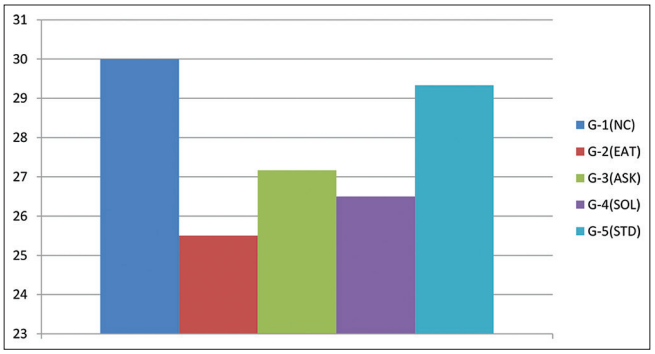

Figure 3: Effect of mean survival time

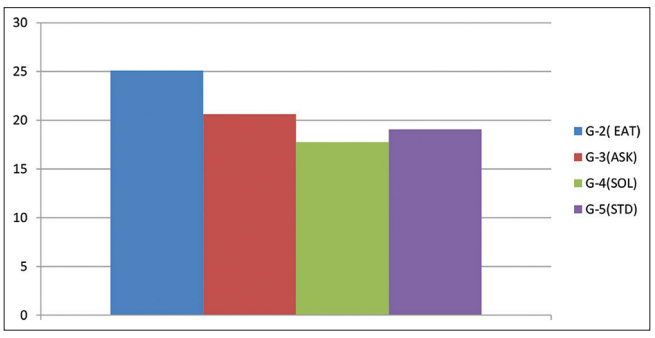

Figure 4: Tumor weight

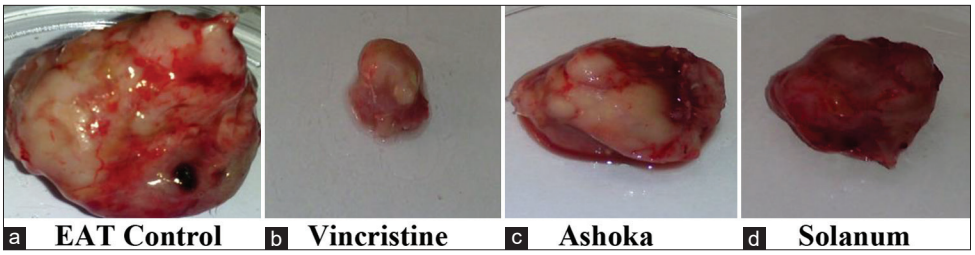

Figure 5: Diagramatic representation of tumor mass of Ehrlich ascites tumor (EAT)-bearing mice: (a) Figure represents the highly developed tumor mass of EAT control group followed by (b) significant reduction in the tumor mass of vincristine (0.5 mg/kg) treated group likewise drugs (Ashoka and Kantkari) treated groups. (c) ASK 400 mg/kg (d). SOL 200 mg/kg

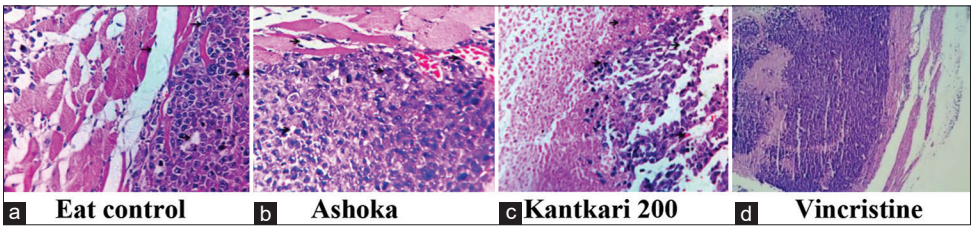

Figure 6: Histopathological analysis of tumor sections of Ehrlich ascites tumor (EAT)-bearing mice: (a) Microphotograph showing tumor infiltration, necrosis, invading cords of malignant cells, and sheets of tumor cells arrows indicate mitotic figures and apoptotic cells in EAT control group (hematoxylin and eosin, H&E 400). (b) Microphotograph depicting inflammatory reaction after treatment with ASHOKA 400 mg/kg/d followed by infiltration of tumor cells and destroyed muscle fibers arrows indicate mitotic figures and apoptotic cells (H&E 400). (c) Microphotograph KANTKARI 200 mg/kg/d showing smooth margins of tumor without any significant evidence of infiltration into surrounding host tissue followed by reduced mitotic figures and increased apoptotic cells arrows indicate mitotic figures and apoptotic cells (H&E 400)

Table 6: Semi-quantitative analysis of histological staining of tumor tissues (*n*=1)

Group	Tumor infiltration at margin	Inflammatory cell infiltration at edge	Mitotic index (no. of MF in 10 HPF)	Apoptotic index (no. of Abs in 10 HPF)
EAT CONTROL	+++	+	7	-
KANTKARI	−/+	+++	2	3
VINCRISTINE	−/+	++	1	5

−/+: No staining, +: Weak, ++: Moderate, +++: Severe. MF: Mitotic figure, HPF: High-power field, Ab: Apoptotic bodies, EAT: Ehrlich ascites tumor

KANTKARI 200 showed moderate staining in tumor section followed by VINCRISTINE which showed strong staining.

Brown staining of VEGF showed angiogenesis in tumor sections. KANTKARI 200 showed less immunoreactivity and no positive cells after staining.Whereas, EAT control strong staining means various positive cells were over 70% which indicate that this group VEGF expression is higher than the other groups [Figures 7 and 8, Table 7].

DISCUSSION

The present study explored the possible anti-tumor properties of drugs (Ashoka and Kantkari) against Ehrlich ascites solid tumor mice model. The Ehrlich ascitic tumor implantation induces a local inflammatory reaction, with increasing vascular permeability, which results in an intense edema formation, cellular migration, and tumor growth.[8] During last few years, apoptosis has become major target in oncology research, molecule-induced apoptosis could be helpful in various cancer treatment.[9] Drugs (Ashoka and Kantkari) induced that a apoptosis was checked against P-815 cells by analyzing endonucleolytic DNA cleavage.[10] Apoptosis induced by drugs (Ashoka and Kantkari) increased caspase-3 mediated cleavage of PARP to generating an 85 kDa fragment. Results of this present study depicted that drugs (Ashoka and Kantkari) also upregulated expression of caspase-3 at the dose of 400 mg/kg to 200 mg/kg, respectively.

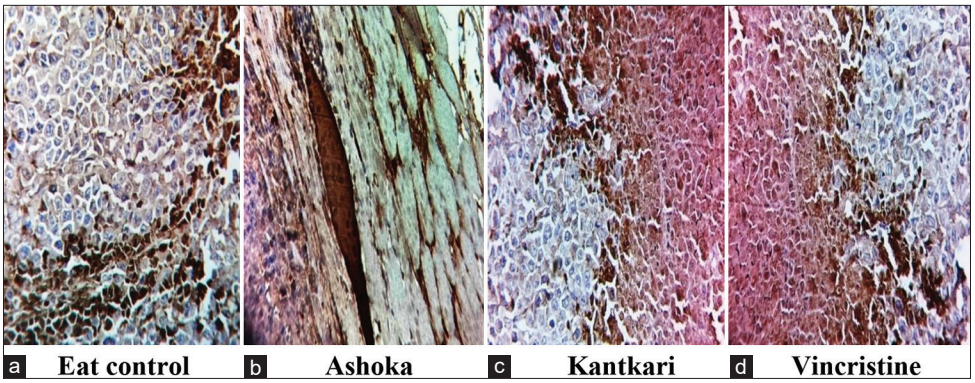

Figure 7: Microphotograph showing immunohistochemical staining of Caspase-3 in tumor section (n = 1). All microphotographs are original magnification; ×400. Scale 20 µM. ASHOKA 400: KANTKARI 200 mg/kg/d; ASHOKA 400: KANTKARI 200 mg/kg/d

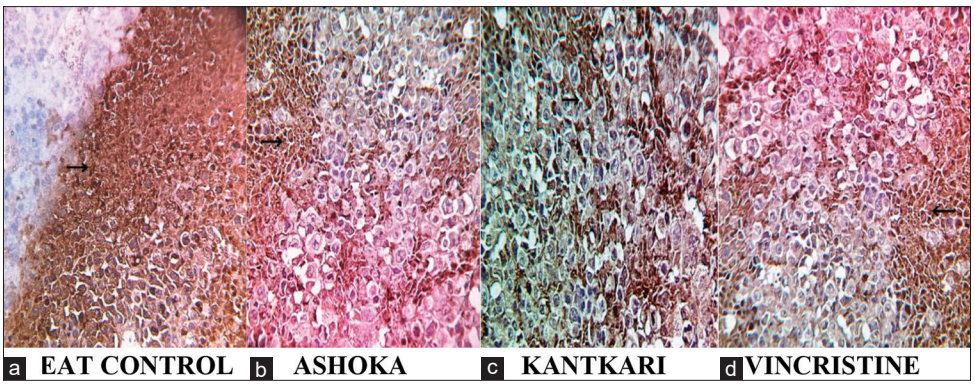

Figure 8: Microphotograph showing localization of vascular endothelial growth factor antigen (brown staining) in tumor section (n = 1). All microphotographs are original magnification; ×400. Scale 20 µM. Vincristine 0.5: Vincristine 0.5 mg/kg/d; Kantkari 200: Kantkari 200 mg/kg/d

Table 7: Semi-quantitative analysis of immunohistological staining of tumor tissues for VEGF and Caspase-3 (n=1)

Group	Caspase-3	VEGF
EAT CONTROL	−/+	+++
VINCRISTINE	+++	−/+
KANTKARI	++	−/+

−/+: No staining, +: Weak staining, ++: Moderate staining, +++: Severe staining. EAT: Ehrlich ascites tumor, VEGF: Vascular endothelial growth factor

Many *in vivo* experimental studies have demonstrated that tumor growth and metastasis are dependent on angiogenesis. Increased neovasculature may allow not only an increase in tumor growth but also enhances hematogenous tumor embolization. Thus, inhibiting tumor angiogenesis may halt the tumor growth and decrease metastatic potential of tumors. Drugs (Ashoka and Kantkari) 400mg/kg to 200 mg/kg, respectively, suppress the VEGF expression by showing no positive cells (−/+) as compare to EAT control. It is well known that ascites tumor growth including EAC cells are angiogenesis dependent. Drugs (Ashoka and Kantkari) downregulated the expression VEGF and prevented the formation of tumor directed capillaries which resulted in reduced angiogenesis in EAC tumor-bearing mice. Previous studies were revealed that Ashoka has anti-proliferative properties on non-small cell lung cancer cells, A549, chronic myeloid leukemia cells, K562, Hep-2 cells, murine B16 melanoma cells, and human metastatic breast cancer cells, MDA-MB231.

Drugs (Ashoka and Kantkari) have showed anti-tumor effect against p815 tumor-bearing DbA-2 mice. Oral administration (gavage) of drugs (Ashoka and Kantkari) dissolved in vegetable oil for 7 days at a dose of 50 and 100 mg/kg/d significantly reduced the tumor volume in comparison with the negative control.[10] One study has proved Ashoka as anti-carcinogenic agent in albino Wistar rats against 3,

4-benzopyrene (B[a] P) induced carcinogenesis. Animals treated with B[a] P in presence of Ashoka developed malignant tumors in 70% of cases at the site of injection. Mean survival time (in days) is higher in Ashoka treated group as compared with control group.[11] Inhibitory effects of Drugs (Ashoka and Kantkari) were observed against DMBA-induced pulmonary tumorigenesis in rats. These *in vivo* studies supported our results by showing decrease in body weight and solid tumor volume. Kantkari 200 mg/kg group exhibited significant reduction in mean body weight against EAT-bearing mice.

Maximum reduction in solid tumor volume was observed with Kantkari (200 mg/kg) which was found significant as compared to disease control group. The %TVI caused by various concentrations of drugs (Ashoka and Kantkari)) were recorded to be 46.36% and 45.22% at a dose of 400 and 200 mg/kg/d, respectively. These figures supported the hypothesis that by reduced body weight and solid tumor volume by drugs (Ashoka and Kantkari)[10] would lead to inhibition of EAT tumor cells *in vivo.* Minimum average tumor weight was found which was result of treatment with Kantkari 200 mg/kg. It would be depicted that Kantkari treatment was proven to reduce the tumor burden in EAT-bearing mice which depicted that Kantkari 200 mg/kg showed that smaller tumor lumps could be related to the slightly increase mean survival time of Kantkari-treated groups. Highest observed percent increase in life span was 97.76% with vincristine 0.5 mg/kg.

All these results depicted that oral administration of drugs (Ashoka and Kantkari) at a dose of 400 mg/kg to 200 mg/kg, respectively, found to be potent anti-tumor agent. These observations, in correlation with the previous findings involving drugs (Ashoka and Kantkari), have shown anti-cancer properties *in vitro*. This present studies explored the anti-angiogenic and anti-apoptosis property of drugs (Ashoka and Kantkari) by evaluating molecular markers. Thus, we strongly suggest that these plants having anti-tumor constituents and after their isolation they may be beneficial for the treatment of cancer that will be matter of further research.

CONCLUSION

The present study was focused on evaluation of anti-tumor properties of drugs (Ashoka and Kantkari) against Ehrlich ascites solid tumor mice model. Among all the doses of drugs (Ashoka and Kantkari) screened in this study 400 mg/kg to 200 mg/kg, respectively, was found to be most efficacious and potent. Kantkari 200 mg/kg significant effect on body weight changes significantly reduced the solid tumor volume in EAT-bearing mice. Mean survival time is increased with Ashoka (400 mg/kg) and Kantkari (200 mg/kg) treated group as compared to EAT control. Reduced expression of VEGF indicated that Kantkari 200 mg/kg possessed anti-angiogenic properties. Caspase-3 is a molecular marker for apoptosis. Kantkari 200 mg/kg induced apoptosis in EAT-bearing mice.

REFERENCES

1. Becher OJ, Holland EC. Genetically engineered models have advantages over xenografts for preclinical studies. Cancer Res 2006;66:3355-8.
2. Choi JR, Lee CM, Jung ID, Lee JS, Jeong Y, Chang JH, *et al*. Apigenin protects ovalbumin-induced asthma through the regulation of GATA-3 gene. Int Immunopharmacol 2009;9:918-24.
3. Lee MJ, Wang CJ, Tsai YY, Hwang JM, Lin WL, Tseng TH, *et al*. Inhibitory effect of 12-Otetradecanoylphorbol- 13-acetate-caused tumor promotion in benzo[a]pyrene- initiated CD-1 mouse skin by baicalein. Nutr Cancer 1999;34:185-91.
4. Ahmed H, Chatterjee PB, Debnath KA. Interaction and *in vivo* growth inhibition of Ehrlich ascites tumor cells by Jacalin. J Biosci 1988;13:419-24.
5. Chaudhary A, Sutaria DK, Huang Y, Wang J, Prabhu S. Chemoprevention of colon cancer in a rat carcinogenesis model using a novel nanotechnology-based combined treatment system. Cancer Prev Res (Phila) 2011;4:1655-64.
6. Bairy KL, Sanath S, Jagetia GC, Somayaji SN, Vidyasagar MS, Baliga MS. Evaluation of intraperitoneal vincristine in malignant peritoneal effusion. Indian J Physiol Pharmacol 2003;47:270-8.
7. Agrawal SS, Saraswati S, Mathur R, Pandey M. Cytotoxic and anti-tumor effects on Ehrlich ascites and human cancer cell line. Life Sci 2011;89:147-58.
8. Zeytinoglu H, Incesu Z, Baser KH. Inhibition of DNA synthesis by carvacrol in mouse myoblast cells bearing a human N-Ras oncogene. Phytomedicine 2003;10:292-9.

9. Shimkin MB, Stoner GD. Lung tumors in mice: Application to carcinogenesis bioassay. Adv Cancer Res 1975;21:1-58.
10. Jaafari A, Mouse HA, M'bark LA, Tilaoui M, Elhansali M, Lepoivre M, *et al*. Differential antitumor effect of essential oils and their major components of *Thymus broussonettii*: Relationship to cell cycle. Herba Polon 2009;55:2.
11. Karkabounas S, Kostoula OK, Daskalou T, Veltsistas P, Karamouzis M, Zelovitis I, *et al*. Cell and Molecular Biology: Concepts and Experiments. New Jersey: John New Jersey, Wiley and Sons; 2008. p. 653-57.