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EVALUATION OF IN VIVO ANTICANCER ACTIVITY OF SALAI GUGGUL EXTRACT IN COMBINATION WITH EPIRUBICIN AGAINST HUMAN HEPATOCELLULAR CARCINOMA

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ABSTRACT

Hepatocellular carcinoma (HCC) is the third leading cause of cancer deaths and the sixth most prevalent cancer worldwide. Liver cirrhosis is the most common risk factor for HCC. Curative treatments include liver resection, ablation, and transplantation, but the 5-year survival rate remains around 50%. Surgical options are limited to patients with small, single tumors and functional livers, while liver transplantation is restricted by donor shortages and high costs, leading most patients to rely on chemotherapy. However, HCC is highly resistant to systemic therapies, and its incidence rate nearly equals its mortality rate. This highlights the need for research into alternative or combination therapies with higher specificity and safety. The in vivo anticancer activity was assessed using an orthotopic xenograft model in C57BL/6 mice. Both extracts, at doses of 50 and 100 mg/kg, significantly reduced tumor weight and volume and increased caspase-3 activity. Survival analysis showed improved mean survival time and lifespan, with combination therapy providing better efficacy and tolerability than single-drug treatments. The results suggest that Salai Guggul extracts enhance the anticancer effects of Epirubicin, offering a promising therapeutic strategy for HCC.

Keywords *– In vivo study, Anticancer activity, Hepatocellular carcinoma, Cancer, Salai Guggul.*

1. INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most prevalent and lethal forms of cancer worldwide, accounting for a significant proportion of cancer-related deaths [1]. Traditional chemotherapy agents such as Epirubicin have been commonly used in the treatment of HCC, but their effectiveness is often limited by severe toxicity and adverse side effects, which necessitates the exploration of novel therapeutic strategies to enhance efficacy and reduce toxicity [2].

Salai Guggul, derived from the gum resin of *Boswellia serrata*, has been traditionally used in Ayurvedic medicine for its anti-inflammatory and analgesic properties [3]. Recent studies have also highlighted its potential anticancer activity, attributed to its active components such as boswellic acids [4]. These components have shown to induce apoptosis and inhibit angiogenesis in various cancer cell lines [5,6]. However, the anticancer potential of Salai Guggul extracts in combination with established chemotherapeutic agents like Epirubicin has not been extensively studied.

This research aims to evaluate the in vivo anticancer activity of Salai Guggul extract in combination with Epirubicin against human hepatocellular carcinoma using an orthotopic xenograft model in C57BL/6 mice. The primary objective is to determine whether

the combination therapy can enhance the efficacy of Epirubicin and reduce its toxicity, providing a basis for the development of more effective and tolerable treatment regimens for HCC.

2. MATERIALS AND METHODS

2.1 Materials

Salai Guggul gum was procured from the local market and authenticated by a botanist and the Department of Botany. Doxorubicin was obtained as a gift sample from Dabur Pharma Ltd., and Silymarin was received as a gift sample from Dabur India Ltd. Standard boswellic acid was provided as a gift sample from Sami Labs, India. All the other chemicals and reagents used were of Analytical grade.

2.2 Preparation and Standardization of Salai Guggul Extract

2.2.1 Methanolic Extract of Boswellia serrata

A 50 g sample of Boswellia gum was finely ground using a mortar and pestle. The powdered gum was then mixed with 950 mL of methanol in a 500 mL round bottom flask and refluxed for two hours. The solvent was filtered to obtain the methanolic extract (Part A). This process was repeated twice more to produce Part B and Part C. All three extracts (Parts A, B, and C) were combined to form the total methanolic extract. The combined methanolic extract was then concentrated using a rotary evaporator at temperatures below 90ºC. The residue obtained was weighed to calculate the percentage yield.

2.2.2 Isolation of Boswellic Acid-Rich Fraction

Ten grams of the methanolic extract prepared as described above was suspended in 50 mL of water. With constant stirring, 20% aqueous KOH was added until the pH reached 9-10, dissolving the extract. The alkaline solution was filtered to remove any precipitate and washed three times with an equal volume of hexane. The solution was then acidified with concentrated HCl, adjusting the final pH to 2, resulting in the formation of a precipitate. This precipitate was filtered, washed with water until neutral, air-dried, and weighed.

2.3 In-vivo Anticancer Activity

2.3.1 Animals

C57BL/6 mice were used for the study. Animals were obtained from Central Animal House Facility (CAHF) of Jamia Hamdard and maintained under pathogen-free conditions. All animals were fed a standard pellet diet ad libitum and housed at 20-25°C under a 12 h light/dark cycle throughout the experiment. Study protocol was approved by institutional ethics committee. All animals received humane treatment and study was conducted in accordance with the guidelines of Institutional Ethics Committee of Jamia Hamdard. Mice were used at 3 days after birth (newborn).

2.3.2 Orthotopic xenograft model

Mice were used as recipients for the orthotopic implantation of human HCC cells. Animals were given HepG2 intrahepatic injection (except for control group). Tumor cell suspension (2X106 cells/20µl) were injected into the livers of recipient mice with a 27- gauge glass syringe with lidocaine as a local anesthetic. The needle was injected at a 30- degree angle into the capsule of the left-lateral hepatic lobe at a depth of 3–5 mm below the skin surface. The needle was then carefully removed and a small piece of

sterile gauze was placed on the injection site to reduce bleeding. The animals were left untreated for the tumors to develop. During this period animals were observed daily.

2.3.3 Treatment Schedule

4 weeks after intrahepatic injection animals were divided into following groups as per the treatment schedule as follows:

Table 1. Treatment schedule of different groups for evaluation of in vivo anticancer activity in orthotopic xenograft model (n=5).

S. No.	Group	Treatment
1.	Tumor Control	Normal saline for 3 weeks in xenograft mice
2.	BS Low	*Salai Guggul* extract (50 mg/kg/day, p.o.) for 3 Weeks xenograft mice
3.	BS High	*Salai Guggul* extract (100mg/kg/day, p.o.) for 3 weeks In xenograft mice
4.	DOX	Epurubicin (2 mg/kg/week i.p.) for 3 weeks in xenograftmice
5.	BS Low+DOX Low	Epurubicin (1 mg/kg/week i.p.) + *Salai Guggul* extract (50mg/kg/day, p.o.) for 3 weeks in xenograft mice

**p.o.: per oral administration; i.p.: intraperitoneal*

After 21 days of study animals from each group was sacrificed by exposure to a lethal dose of anesthetic in a desiccator. After dissection and removal of the tissues, it was washed in sterile PBS and further, biochemical, histopathological and tumor evaluations were carried out.

For assessment of mean survival time (MST), percentage increment of life span (%ILS), groups were separately maintained. The animals from each group were kept on standard pellet diet and water ad libitum.

2.4 Caspase-3 Activity

Caspase-3 activity was measured in hepatic tissue, using capase-3/CPP32 colorimetric assay kit (Bio Vision, Inc., USA. Briefly, tissue homogenate for each sample was prepared using chilled lysis buffer and incubated in ice for 10 min followed by centrifugation for one min at 10,000 g. After centrifugation, the supernatant obtained was collected. To the supernatant 50 µL of 2X reaction buffer containing 10 mM DTT was added. To this, 5.0 µL of 4.0 mM DEVD-pNA substrate solution was added and incubated at 37°C for two h and absorbance was read at 405 nm.

2.5 Tumor regression parameters Tumor weight

Tumor weight was calculated by directly weighing the tumors removed at the end of the experiment.

2.6 Tumor volume

The two tumor diameters (shortest and longest diameters) were calculated with the help of a digital vernier caliper. Further, tumor volume was calculated from these lengths using the following formula:

2.7 Statistical analysis

Values of different parameters were expressed as mean ± standard deviation (SD). Statistical analysis of data was carried out by one-way analysis of variance (ANOVA) followed by Tukey's test using GraphPad® software. All the treatment groups were compared with each other and value of $P < 0.05$ were considered as statistically significant.

3. RESULT AND DISCUSSION

3.1 In-vivo anticancer activity

In-vivo anticancer activity of the *salai guggul* extracts alone as well as in combination with epirubicin was evaluated in orthotopic implant model developed using C57BL/6 mice. The pups were injected with HepG2 hepatocellular carcinoma cells and tumors were allowed to develop for four weeks after which, animals were divided into various groups and treated with single and combination treatments for the representative tumours from various treatment groups are shown in Fig. 1. The orthotopic xenograft generated white nodular tumors with well-defined limits in the hepatic lobes of the newborn mice.

3.2 Tumor regression parameters

Tumor weight was recorded immediately after removing the tumors. Tumor weight in animals' xenograft which were treated with normal saline was 161.0 ± 9.67 mg. Tumorweight in group treated with 50mg/kg of *salai guggul* extract (BS low dose) was 144.0± 4.85 mg which was significantly less than the tumor control group ($P < 0.05$ vs tumorcontrol) (Fig. 1). Tumor weight in animals treated with 100mg/kg of *salai guggul* extract (BS high dose) was 122.8 ± 7.49 mg which was even at significantly lower level($P < 0.001$) compared to tumor control group. The tumor weight in animals treated withhigh dose were comparable to group treated with 2mg/kg dose of epirubicin ($P>0.05$). It was observed that animals treated with combination of 1mg/kg epirubicin with 50mg/kg of *salai guggul* extract showed significantly ($P < 0.001$) less tumor weight than single agent treatments with *salai guggul* extract at 50 and 100mg/kg respectivelyas well as with epirubicin at 2mg/kg dose.

Tumor volume was calculated based on the lengths of the excised tumors. Tumor volume in three single treatment groups i.e. salai guggul extract 50mg/kg (BS low dose), 100mg/kg (BS high dose) and epirubicin 2mg/kg (EPU) were significantly reduced ($P < 0.001$) as compared to tumor control groups (Fig. 2). Tumor volume in these three treatment groups showed insignificant difference when compared with each other ($P>0.05$). However, concomitant treatment of salai guggul extract 50 and epirubicin 1mg/kg further reduced tumor volume to even lower ($P < 0.001$ vs salai guggul 50mg/kg, 100mg/kg and epirubicin 2mg/kg respectively).

Overall, the tumor inhibition with 50mg/kg doses of salai guggul extracts was 10.56 % (Fig. 3) respectively. Tumor inhibition with higher dose i.e. 100mg/kg of salai guggul extracts were 23.73% respectively. Epirubicin exhibited 26.21% tumor inhibition. However, combination of doxorubicin 1mg/kg with lower dose (50mg/kg) of salai guggul extract produced >50% tumor inhibition whereas combination of same dose of doxorubicin produced 46.96% tumor inhibition.

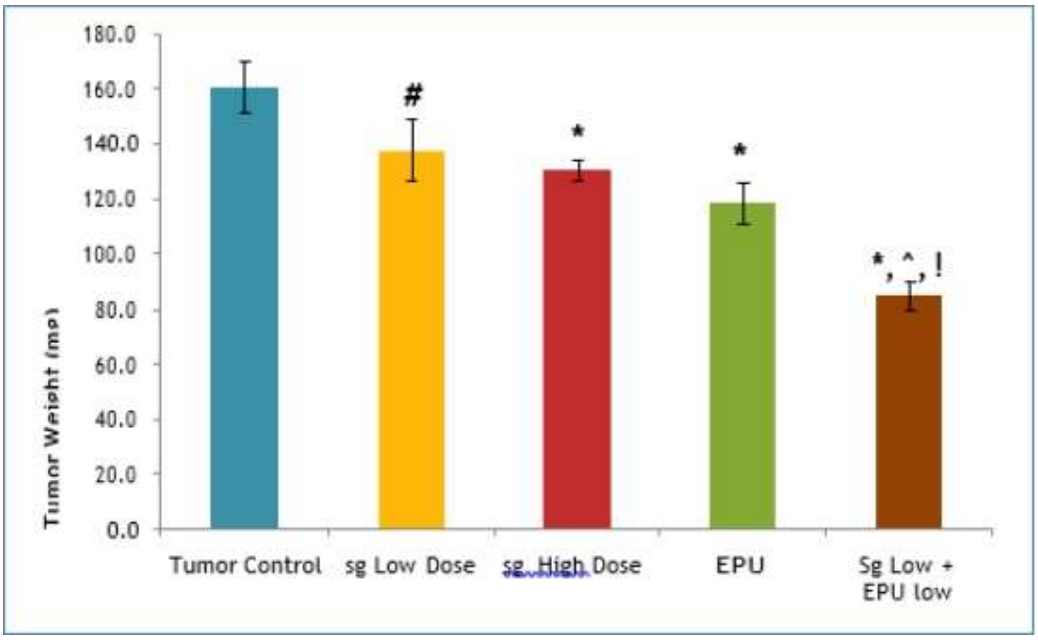

Fig. 1. Showing tumor weight in different treatment groups with reference to B. serrata extract (BS Low dose: salai guggul extract 50mg/kg; BS High dose: salai guggul extract 100mgkg; EPU: epirubicin 2mg/kg; DOX Low: epirubicin 1mg/kg); # P<0.05 vs Control; * P<0.001 vs Tumor Control; ^P<0.001 vs DOX; !P<0.001 vs BS High Dose.

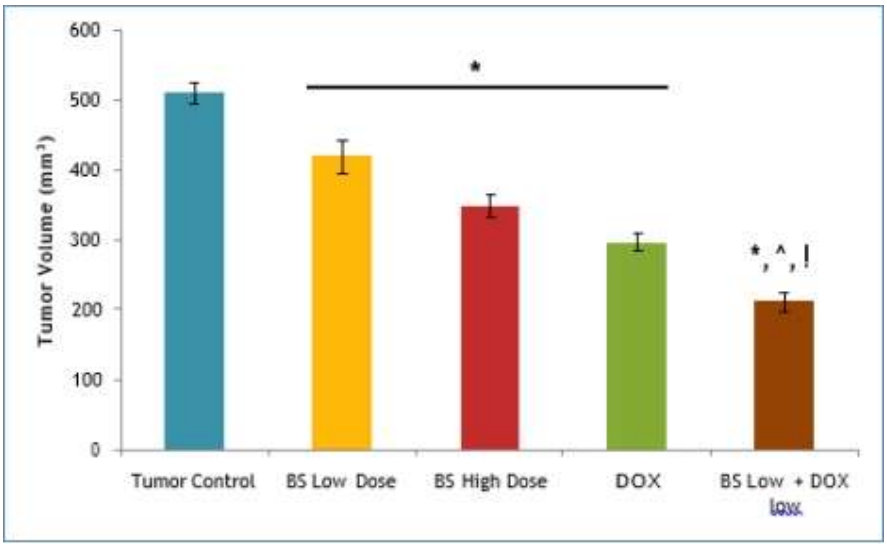

Fig. 2. Showing Tumor volume in different treatment groups with reference to B. serrata extract (BS Low dose: salai guggul extract 50mg/kg; BS High dose: salai guggul extract 100mgkg; EPU: epirubicin 2mg/kg; EPU Low: epirubicin 1mg/kg); * P<0.001 vs Tumor Control; ^P<0.001 vs EPU; !P<0.001 vs BS High Dose.

3.3 Caspase-3 activity

Caspase-3 activity was evaluated as a marker for apoptosis in the xenografted animals. Results are shown in Fig. 4 and Fig. 5. Treatment with salai guggul extract at the dose levels of 50mg/kg (SG low dose) and 100 mg/kg (BS high dose) significantly (P <0.001 vs. tumor control) increased caspase-3 activity around 1.9 and 3 fold respectively. Further, treatment with 50mg/kg of salai guggul extract in combination with 1mg/kg of epirubicin caused 4.6 fold increase in caspase-3 activity which was

significantly (P < 0.001) better than the caspase-3 activity observed in groups treated with either 100mg/kg of salai guggul extract or 2mg/kg of epirubicin.

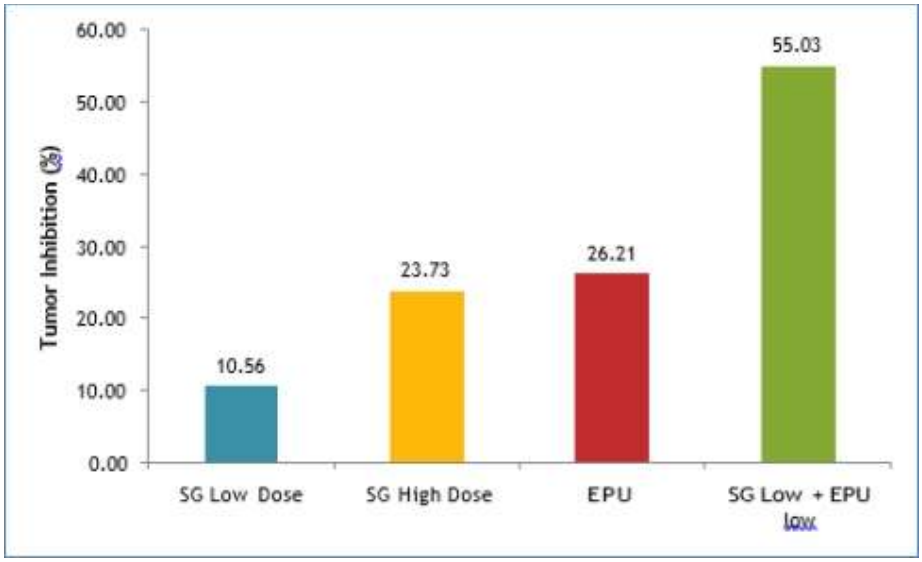

Fig. 3. Showing percentage tumor inhibition in different treatment groups with reference to salai guggul extract (BS Low dose: salai guggul extract BS High dose: B. serrata extract 100mgkg; EPI: epirubicin 2mg/kg; EPU Low: epirubicin 1mg/kg).

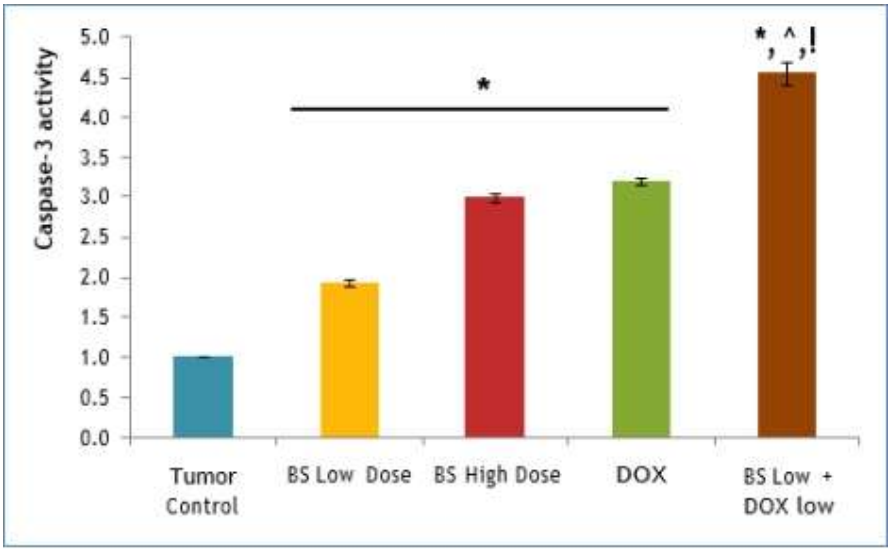

Fig. 4. Showing caspase-3 activity in different treatment groups with reference to salai guggul extract (SG Low dose: salai guggul extract 50mg/kg; SG High dose: salai guggul extract 100mgkg; EPU: epirubicin 2mg/kg; DOX Low: epirubicin 1mg/kg); * P<0.001 vs Tumor Control; ^P<0.001 vsDOX; !P<0.001 vs BS High Dose.

3.4 Dose Survival parameters

Survival of animals was assessed through calculation of mean survival time (MST). Survival of the animals treated with 50mg/kg dose of extracts of salai guggul (Fig. 6) and increased from 24.75 ± 3.36 days respectively. In case of salai guggul extract this increase was found to be significantly better (P < 0.05 vs Tumor control). However, higher dose (i.e. 100mg/kg) of both salai guggul extracts further significantly (P<0.001 vs tumor control) improved the MST of treated animals to 55.5 ± 4.33 8 days

respectively. MST of animals treated with epirubicin at 2mg/kg was 44.87 ± 2.48 days. MST of doxorubicin treated groups was although lower than the MST of groups treated with 100mg/kg dose of either extract but it was statistically comparable (P > 0.05). Treatment group which was given combination of combination of salai guggul extract 50mg/kg along with epirubicin 1mg/kg were found to increase MST to 63.5 ± 3.36 days. Similarly, group treated with combination of salai guggul extract 50mg/kg and doxorubicin 1mg/kg increase MST to 58.65 ± 3.19 days. In both these cases of combination treatment, MST values were significantly better (P < 0.05) than epirubicin treated groups (EPU).

Based on these values of MST, percentage increase in life span (%ILS) of various treatment groups were calculated. Average increase in life span of groups treated with 50mg/kg and 100mg/kg of salai guggul extracts were 159.59% and 224.24% days respectively (Fig. 6). Increase in life span of animals given 50mg/kg and 100mg/kg of was 139.89% respectively (Fig. 7). Whereas the combination treatment of doxorubicin with respective lower doses of salai guggul extracts were 256.57%.

The xenografted animals of various treatment groups were also subjected to Kaplan– Meirer's survival analysis. Results showed that treatment with both 50mg/kg and 100mg/kg doses of both salai guggul extracts improved survival of animals from 10 to approximately 20 days, respectively whereas combination of epirubicin either salai guggul extracts improved survival to 45 days respectively.

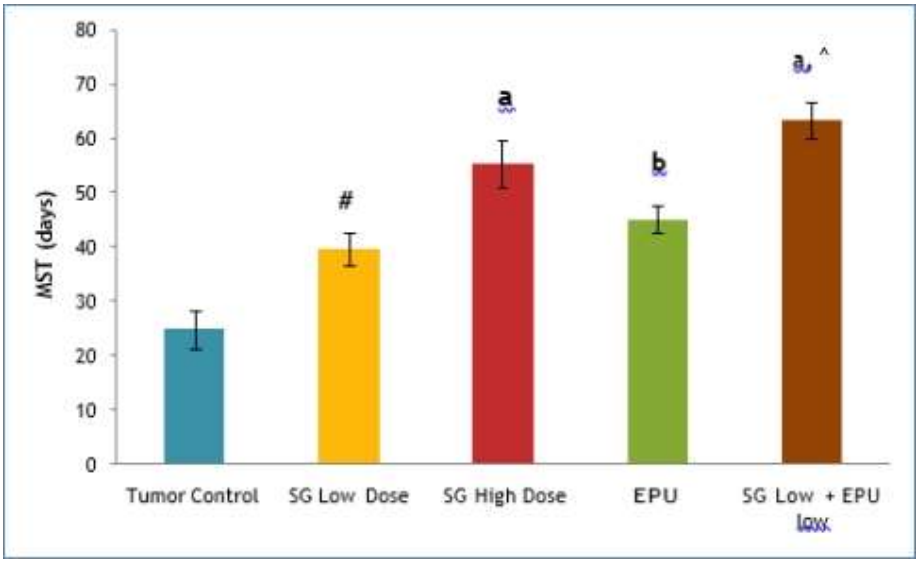

Fig. 5. Showing mean survival time (MST) of different treatment groups with reference to salai guggul extract (SG Low dose: salai guggul extract 50mg/kg; BS High dose: salai guggul extract 100mgkg; EPU: epirubicin 2mg/kg; DOX Low: epirubicin 1mg/kg); # P<0.05 vs Tumor Control; a P<0.001 vs Tumor Control; b P<0.01 vs Tumor Control; ^ P<0.01 vs EPU.

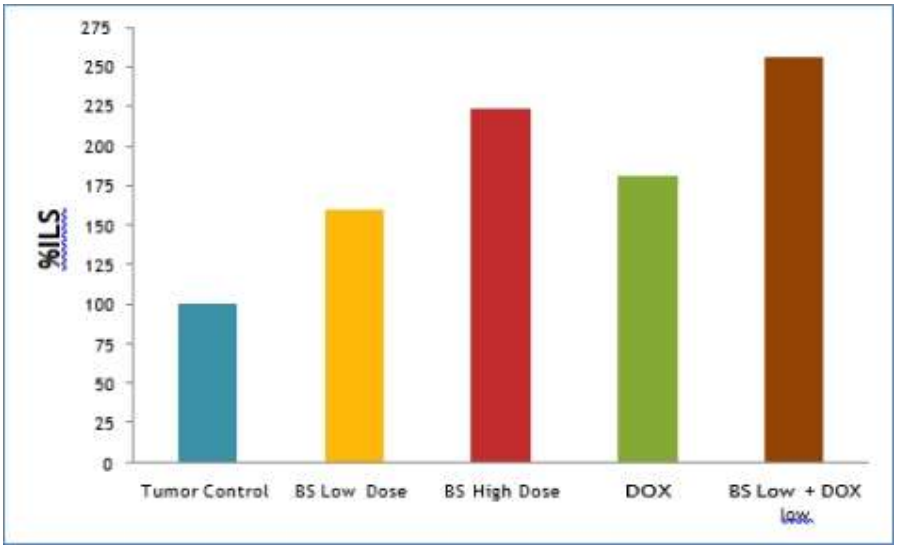

Fig. 6. Showing percentage increase in life span (%ILS) of different treatment groups with reference to salai guggul extract (SG Low dose: salai guggul extract 50mg/kg; BS High dose: salai guggul extract 100mgkg; EPU: epirubicin 2mg/kg; EPU Low: epirubicin 1mg/kg)

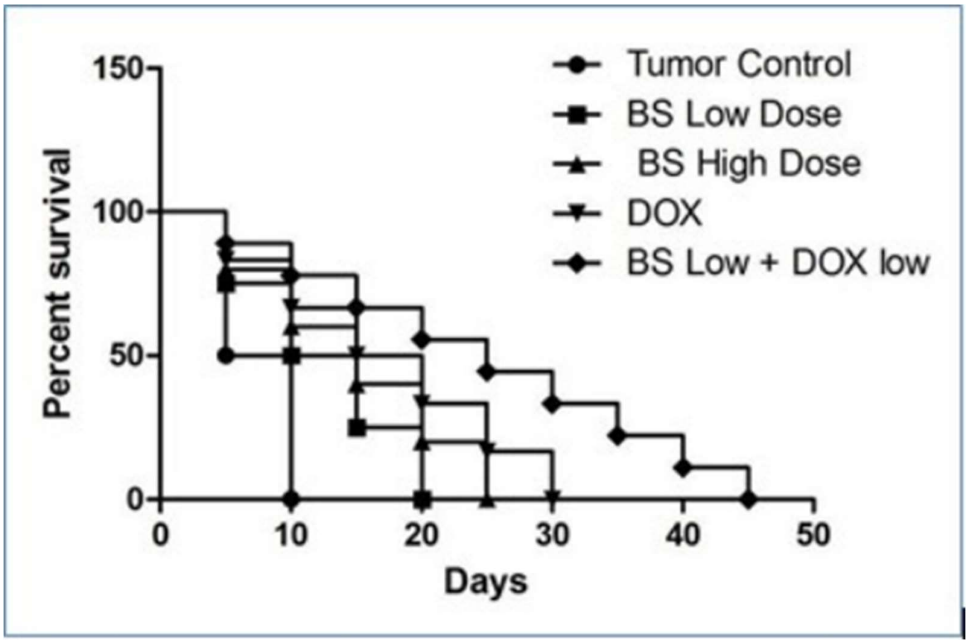

Fig. 7. Kaplan–Meier survival curve for various treatment groups with reference to salai guggul extract showing percentage survival of animals vs. no. of days. (SG Low Dose: salai guggul extract 50mg/kg; BS High Dose: salai guggul extract 100mgkg; EPU: epirubicin 2mg/kg; EPU Low: epirubicin 1mg/kg).

4. CONCLUSION

The results from the study of anticancer potential against the orthotopic xenograft model in C57BL/6 mice indicate a promising opportunity for both extracts to be used as adjuvant or combination therapies with Epirubicin for hepatocellular carcinoma. This represents the first report of such findings and suggests a potential avenue for further drug development.

The extracts were found to enhance the efficacy of Epirubicin while improving tolerability by reducing its toxicity. Further investigation into the molecular mechanisms of action of Salai Guggul extracts could pave the way for the development of improved therapeutic regimens for hepatocellular carcinoma in the future.

5. REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69-90.
2. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet. 2003;362(9399):1907-17.
3. Ammon HP. Boswellic acids in chronic inflammatory diseases. Planta Med. 2006;72(12):1100-16.
4. Poeckel D, Werz O. Boswellic acids: biological actions and molecular targets. Curr Med Chem. 2006;13(28):3359-69.
5. Liu JJ, Nilsson A, Oredsson S, Badmaev V, Duan RD. Boswellic acids trigger apoptosis via a pathway dependent on caspase-8 activation but independent on Fas/Fas ligand interaction in colon cancer HT-29 cells. Carcinogenesis. 2002;23(12):2087-93.
6. Park YS, Lee JH, Harwalkar JA, Bondar J, Safayhi H, Golubic M. Acetyl-11-keto-β-boswellic acid (AKBA) inhibits lipopolysaccharide-mediated cyclooxygenase-2 gene expression in human monocytes by blocking p38 MAPK and NF-κB pathways. J Biol Chem. 2002;277(4):3373-80.