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Evaluation of anticancer efficacy of BOSWELLIA SERRATA's Extract against hepatocellular carcinoma

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ABSTRACT
This paper is about Evaluation of anticancer efficacy of BOSWELLIA SERRATA's Extract against
hepatocellular carcinoma. The effect of B. serrata in combination with doxorubicin was investigated and analysed the interaction using Isobolographic method. cytokine estimation was carried out to find out the effect of test drugs on apoptosis pathway in HepG2 and Hep3B cell lines. In first stage the extracts i.e of B. serrata was subjected to in vitro studies for evaluation of cytotoxicity using MTT assay as well as investigation of their apoptotic action using cytokine assay. Study was carried out in HepG2 and Hep3B hepatocellular carcinoma cell lines. the test drugs i.e B. serrata exerted antiproliferative effect on HepG2 and Hep3B cells. in second stage, in-vivo experiment was carried out to find out the effect of B. serrata extracts on doxorubicin induced hepatic toxicity in Wistar rats. third stage of the study involved evaluation of in vivo anticancer activity using orthotopic xenograft implant model developed in C57BL/6 mice.

I. INTRODUCTION

Hepatocellular carcinoma

A surprising role in preserving the body's functionality and managing homeostasis is played by the liver, one of the body's major organs and the principal site of intense metabolism and excretion. It participates in almost every metabolic process that results in development, the prevention of disease, the transport of nutrients, the creation of energy, and reproduction. The liver also plays a crucial role in the host's ability to fight against infections. By removing bacteria from the portal and systemic circulation, it also produces humoral substances that stimulate white blood cell activity and bacterial death.

Hazard variables and epidemiology

Numerous chronic liver conditions frequently result in hepatocellular carcinoma. Age-adjusted incidence of HCC varies from 55 to 149 per million people yearly, with an incidence of 600,000–1,000,000 fatalities. Some regions, including East, South-East Asia, and sub-Saharan Africa, have hepatocellular carcinoma rates that are higher than those in the western world. However, through epidemiological research, a variety of HCC risk factors have been discovered. Liver cirrhosis is the primary cause of HCC in western countries, which highlights the significant influence that disease frequency has on HCC development. The third or fourth most common cancer in the world right now is HCC. Viral infections, aflatoxin exposure, alcoholism, and hereditary conditions including hemochromatosis and diabetes are also significant risks for HCC.

Viral Hepatitis B

Throughout the world, chronic hepatitis B virus (HBV) infection is the main cause of HCC. This is further illustrated by the similarity between the geographic distribution of HCC and that of HBV. By integrating its DNA into the host's genome, the single-stranded DNA virus HBV may activate proto-oncogenes or repress growth-regulating genes. The HBx protein, which is produced by the HBV X gene, is essential for the development of hepatocarcinogenesis because it regulates the expression of tumour suppressor genes like p53 and APC as well as cellular oncogenes like c-myb and c-myc in primary hepatocytes. HBx can also have an antiapoptotic effect by causing Raf-1 kinase to translocate into the mitochondria.

HepatitisCvirus

In order to cause hepatocarcinogenesis, the RNA virus known as the hepatitis C virus (HCV) interacts with host-viral protein. Although

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the mechanism is yet unknown, it is widely accepted that HCV infection and the development of HCC are related. HCV core, NS5A, NS4B, and NS3 are at least four of the HCV gene products that clearly show changes in a number of possibly carcinogenic pathways (Liang and Heller, 2004). The HCV core protein controls the transcription of several cellular genes, including the proto-oncogene c-myc, and it also promotes viral persistence by controlling proteins that prevent apoptosis.

Terpenoids as natural HCC inhibitors

Terpenoids are a significant class of natural chemicals. Hemiterpenoids, which have one isoprene unit, monoterpenoids, which have two, sesquiterpenoids, which have three, diterpenoids, which have four, sesqueterpenoids, which have five, triterpenoids, which have six, tetraterpenoids, which have eight, and polyterpenoids, which have more than eight isoprene units with a larger number of isoprene units.

Boswellia serrata: The terpenoids that may be used to treat cancer The Boswellia serrata branched tree can be found in India, Northern Africa, and the Middle East. Historical uses for a sticky oleo-resin made by plant bark include antiarthritic, astringent, stimulant, expectorant, and antibacterial properties. Boswellic acid and its derivatives, such as acetyl-11-keto-boswellic acid, are present in B. serrata. Boswellic acid can be used to treat a variety of chronic inflammatory conditions, including rheumatoid arthritis and atherosclerosis. Studies have demonstrated that boswellic acid and its derivatives block NF-kB signalling. Additionally, it has been shown to bind to inhibit IKKa and IKKb kinases, which changes NF-kB signalling. Boswellic acids may have anticancer capabilities in addition to their anti-inflammatory properties, according to studies on brain tumours and leukemic cells.

II. METHODOLOGY

Materials

B.serrata, Doxorubicin, Sylimarin, Standard boswellic acid and all other chemicals in analytical grade

Method

B. serrata extract preparation and standardisation

Boswellia serrata extract in methanol

A mortar and pestle were used to smash 50 g of weighed boswellia gum. 950 mL of methanol and gum powder were combined and refluxed for two hours in a round bottom flask with a capacity of 500 mL The solvent was filtered twice in order to get the methanolic extract (Part A) (Part B and C). The three extracts' portions A, B, and C were mixed to form the total methanolic extract. Methanolic extract was additionally condensed using Rota vapour at temperatures below 90°C. Weighing the collected residue allowed us to calculate the yield percentage.

Samplepreparation

- The stock solution of standard boswellic acids was prepared by weighing 44.6 mg of the reference standard and combining it with 10 mL of HPLC-grade methanol in a volumetric flask. It was sonicated for 20 minutes.
- B. serrata methanolic extract: 102.1 mg of B. serrata methanolic extract were dissolved in 10 mL of methanol of HPLC grade, and the resulting solution was sonicated for 20 minutes.
- Boswellic acid rich fraction that was isolated was dissolved in 10 mL of HPLC-grade methanol and subjected to sonication for 20 minutes. The solution contained 50.1 mg of the boswellic acid rich fraction.

III. RESULT and DISCUSSION

Pharmacological studies were carried out broadly in three stages. In first stage the extracts (i.e of B. serrata) was subjected to in vitro studies for evaluation of cytotoxicity using MTT assay as well as investigation of their apoptotic action using cytokine assay. Study was carried out in HepG2 and Hep3B hepatocellular carcinoma cell lines. the test drugs i.e B. serrata exerted antiproliferative effect on HepG2 and Hep3B cells. The IC 50 values of B. serrata extract in HepG2 and Hep3B cells were 21.21±0.92 µg/ml and 18.65±0.71 µg/ml, respectively.

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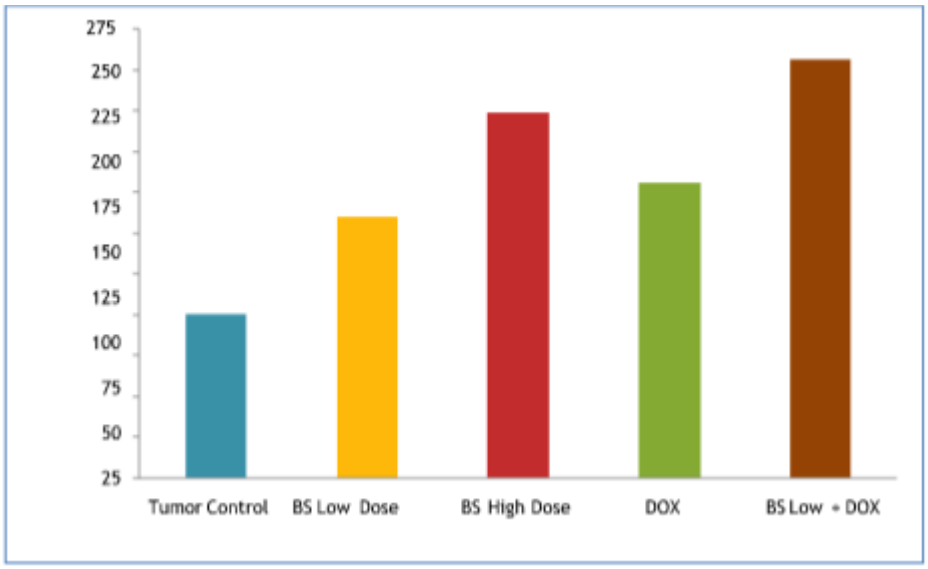

FIG. Showing percentage increase in life span

Doxorubicin has been recommended for chemotherapy of hepatocellular carcinoma by various clinical guidelines. Therefore, we also investigated the effect of B. serrata in combination with doxorubicin and analysed the interaction using Iso-bolographic method. The effect of doxorubicin was found synergistic with B. serrata. Further, cytokine estimation was carried out to find out the effect of test drugs on apoptosis pathway in HepG2 and Hep3B cell lines.

	Control (Group 1)	**Dox (Group 2)**	**Dox+BS250 (Group 3)**	**Dox+BS500 (Group 4)**	**Dox+SY100 (Group5)**
SGPT(IU/L)	83.44±13.01	360.05±17.9	171.56±12.7	169.69±10.2	135.94±8.1
SGOT(IU/L)	57.50±7.72	209.17±9.04	113.01±8.33	106.57±8.94	110.03±9.37
Albumin (mg/dL)	4.54±0.23	1.70±0.32	3.35±0.26	3.43±0.17	3.38±0.11
ALP (IU/L)	136.68±12.3	414.55±15.7	158.98±12.8	155.18±11.1	157.35±13.4
TotalBilirubin (mg/dL)	1.63±0.22	6.68±0.30	4.16±0.22	3.79±0.14	2.95±0.22
MDA (nmoles/mg)	57.51±5.59	332.63±13.0	90.73±4.62	66.81±3.29	64.14±2.43

Table Showing level of various biochemical markers of hepatic toxicity in control, doxorubin intoxicated groups and groups treated with B. serrata extract

Once the effect of B. serrata was established through in vitro anticancer and in vivo hepatoprotective studies, third stage of the study involved evaluation of in vivo anticancer activity using orthotopic xenograft implant model developed in C57BL/6 mice. Both test preparations were administered at two dose levels of 50 and 100mg/kg. Additionally, effect of doxorubicin was also evaluated at 2mg/kg dose for single treatment and 1mg/kg dose in combination with B. serrata extract. Analysis of tumor regression parameters showed that both tumor weight as well as tumor

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volume in xenografterd mice treated with B. serrata extracts at two dose levels of 50 and 100mg/kg were significantly lower than tumor control animals. Significant increase in caspase-3 activity was also observed in treated animals, indicating that in vivo mechanism of the B. serrata extracts were same as
reported in in vitro study. Further, survival parameters of treated animal showed that treatment with both test drugs at 50 and 100mg/kg dose levels significantly improved the mean survival time and life span of the animals. The tumor regression parameters, caspase-3 activity as well as survival parameters in animal groups treated with 100mg/kg of B. serrata extracts were comparable to doxorubicin (2mg/kg) treated group. However, treatment with 1mg/kg doxorubicin in combination with either B. serrata extracts reduced tumor size to almost half and increased lifespan of animals by >200%. Overall results of combination of doxorubicin and any of B. serrata showed better efficacy and improved tolerability than single drug treatments with B. serrata extract doxorubicin.

EVALUATION

The paradigm for the experiment was doxorubicin-induced liver damage. To cause liver damage, a single intraperitoneal dose of 30 mg/kg body weight was administered. For this study, sylimarin was given at the usual dose of 100 mg/kg body weight. Animals were separated into five groups and given different treatments.

For the purpose of estimating serum, blood was drawn from the tail vein 72 hours after doxorubicin injection. The liver was then removed, cleansed with ice-cold saline, and kept for biochemical examination after the animals were slain under vigorous anaesthesia. Each group's liver tissue underwent histological analysis after being fixed in 10% formalin for up to 48 hours

IV. CONCLUSION

- The standardised terpenoid rich fraction of B. serrata was prepared and used for exploration against hepatocellular carcinoma alone and in combination with doxorubicin.
- In vitro assay on HepG2 and Hep3B proved that B. serrata possess antiproliferative and proapoptotic properties, however combination of submaximal dose with doxorubicin may have good future for treatment of hepatocellular carcinoma as proved by Isobolographic analysis.
- Extract possess significant hepatoprotective potential which is comparable to standard drugs silymarin as observed through in vivo studies.
- Results of anticancer potential against orthotopic xenograft model in C57BL/6 mice showed good opportunity of both extracts as adjuvant/combination regimen with doxorubicin for hepatocellular carcinoma, which is the first report and may be explored further for drug development.
- The extracts were found to enhance efficacy of doxorubicin with improved tolerability by reducing its toxicity. Further, investigation into mechanism of action of B. serrata extracts at molecular level can pave the way for development of better therapeutic regimen for hepatocellular carcinoma in future.

REFERENCES

[1]. R. S. Benjamin, P. H. Wiernik, and N. R. Bachur, “Adriamycin chemotherapy: efficacy, safety, and pharmacologic basis of an intermittent single high dosage schedule,” Cancer, vol. 33, no. 1, pp. 19–27, 1974.

[2]. S. Wang, E. A. Konorev, S. Kotamraju, J. Joseph, S. Kalivendi, and B. Kalyanaraman, “Doxorubicin induces apoptosis in normal and tumor cells via distinctly different mechanisms: intermediacy of H 2 O 2 - and P53 -dependent pathways,” Journal of Biological Chemistry, vol. 279, no. 24, pp. 25535–25543, 2004.

[3]. O. Hovorka, V. Subr, D. Veˇtvicˇka et al., “Spectral analysis of doxorubicin accumulation and the indirect quantification of its DNA intercalation,” European Journal of Pharmaceutics and Biopharmaceutics, vol. 76, pp. 514–524, 2010.

[4]. P. Mukhopadhyay, M. Rajesh, S. Ba´tkai et al., “Role of superox- ide, nitric oxide, and peroxynitrite in doxorubicin-induced cell death in vivo and in vitro,” The American Journal of Physiology— Heart and Circulatory Physiology, vol. 296, pp. 1466–1483, 2009.

[5]. Y. Shen, M. Takahashi, H. Byun et al., “Boswellic acid induces epigenetic alterations by modulating DNA methylation in colorectal cancer cells,” Cancer Biology and Therapy, vol. 13, no. 7, pp. 542–552,2012.

[6]. S. P. Yong, J. H. Lee, J. Bondar, J. A. Harwalkar, H. Safayhi, and M. Golubic, “Cytotoxic action of acetyl-
[7]. 11-keto-B-boswellic acid (AKBA) on meningioma cells,” Planta Medica, vol. 68, no. 5, pp. 397–401, 2002.
[8]. Y. Shao, C. Ho, C. Chin, V. Badmaev, W. Ma, and M. Huang, “Inhibitory activity of boswellic acids from
[9]. Boswellia serrata against human leukemia HL-60 cells in culture,” Planta Medica, vol. 64, no. 4, pp.328–331, 1998.
[10]. L. Zhao, M. G. Wientjes, and J. L.-. Au, “Evaluation of combina- tion chemotherapy: integration of nonlinear regression, curve shift, isobologram, and combination index analyses,” Clinical Cancer Research, vol. 10, no. 23, pp. 7994–8004, 2004.
[11]. J. J. Liu, A˚ . Nilsson, S. Oredsson, V. Badmaev, W. Z. Zhao, and R. D. Duan, “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, vol. 23, no. 12, pp. 2087–2093, 2002.
[12]. X. Pang, Z. Yi, X. Zhang et al., “Acetyl-11-keto-B-boswellic acid inhibits prostate tumor growth by suppressing vascular endothelial growth factor receptor 2-mediated angiogenesis,”Cancer Research, vol. 69, no. 14, pp. 5893–5900, 2009.
[13]. S. Bhushan, A. Kumar, F. Malik et al., “A triterpenediol from Boswellia serrata induces apoptosis through both the intrinsic and extrinsic apoptotic pathways in human leukemia HL-60 cells,” Apoptosis, vol. 12, no. 10, pp. 1911–1926, 2007.
[14]. R. Kirk, “Targeted therapies: the maths behind combination therapy,” Nature Reviews Clinical Oncology,
[15]. vol. 10, no. 9, p. 488, 2013.
[16]. W. Yeo, T. S. Mok, B. Zee et al., “A randomized phase III study of doxorubicin versus cisplatin/interferon
[17]. ��-2b/doxorubicin/flu- orouracil (PIAF) combination chemotherapy for unresectable hepatocellular carcinoma,” Journal of the National Cancer Insti- tute, vol. 97, no. 20, pp. 1532–1538, 2005.
[18]. Forner, J. M. Llovet, and J. Bruix, “Hepatocellular carcinoma,”The Lancet, vol. 379, no. 9822, pp. 1245–1255, 2012.