ISSN No: 0976-5921
Published online at https://ijam.co.in

International Journal of Ayurvedic Medicine, Supplement of International Conference on Ayurveda-Yoga-Nathpanth - 2025

Mechanism of *Arkeshwara rasa* to combat hepatocellular carcinoma

Research Article

Remya Jayakumar[1*], Manoj Kumar Dash[2], Rama Chandra Reddy K[3], Namrata Joshi[4]

1. PhD Scholar, 3. Senior Professor, 4. Professor, Department of Rasashastra and Bhaishajya Kalpana, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, UP, India.
2. Associate Professor, Department of Rasashastra and Bhaishajya Kalpana, Shri Narayan Prasad Awasthi Government Ayurved College, Raipur. India.

Abstract

Background: Immunomodulatory treatments along with systemic and loco-regional methodology of treatment approach have reduced the incidence of Hepatocellular carcinoma (HCC) in recent observations. The phytomolecular applications have been seen to reverse the hepatic fibrosis aiding in regeneration of hepatocytes. The cytotoxicity of *Arkeshwara rasa* (AR) was investigated on HCC cell lines to observe the general signs of apoptosis. Methodology: The MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay and Ethidium Bromide/Acridine orange Assay (EtBr/AO) were subjected on AR to know the cytotoxicity and the apoptotic results. Results: The IC_{50} of 37.14 µg/ml was exhibited by AR against Huh 7 cell lines and the (EtBr/ AO) Assay demonstrated the early and late apoptotic features in comparison with Untreated and Doxorubicin standard drug. Conclusion: AR on the basis of present evaluation against HCC cell lines and previous results on various other cell lines might be able to check the pathogenesis progression of HCC cells initiating the apoptotic features or reviving the vulnerable cell from further damage because of the phyto-constituents or mineral inclusions. These preliminary observations have to be again revalidated with reverse transcription, cDNA synthesis, western blotting, cell cycle analysis, etc.

Keywords: *HCC, AR, Rutin, Hesperidine, Kaempferol, Quercetin, MTT Assay, AO Assay*.

Introduction

Hepatocellular carcinoma (HCC) is addressed in the ICD 10 categorization, as the disease with rare incidence in United States and more in Asian countries. The prevalence of Hepatitis also follows such a pattern in Asian countries than the European regions revealing the chances of microbial resistance flaring up the world wide spread. (1) The infections and inflammations in the presence or absence of alcohol as in non- alcoholic cirrhotic conditions subsequently due to chronic nature also end as this carcinoma. (2)The HCC ranks as the sixth in overall cancer incidence, however stands fourth in the mortality turn over. (3) This data is a drop over from the incidence and death rate of 5th and 2nd positions, respectively. (4) This recent rise of survival rate from 18% to 43% has been concluded due to the systemic, immunomodulatory, loco-regional methodology of treatment approach.(5) The most effective treatment comprises the application of Orthotopic Liver Transplantation (OLT) and Marginal resections depending upon the spread and size of the tumor. Even though the asymptomatic phase and the late stage diagnosis delays the treatment aspects and leads to poor prognosis.(6) The multikinase inhibitor-Sorafenib faces the challenge of chemotherapeutic resistance and the main reason lies with ferroptosis phenomenon due to the drug. (7). The irreversible fibrotic change have been found to be generated from transforming growth factor (TGF)-β1 and the NF-κB cell signaling pathways. The same NF-κB protiens are the intermediates in the gut dysbiosis and the inflammatory routes, respectively. Therapies focusing the manipulation of dysbiosis are found benefitting the gut microbiome as well as the inflammatory routes. The Ayurvedic herbal medicine are found to positively dominate these regions like hindering the fibrogenesis, attenuating the oxidative stress and supporting the regeneration of hepatocytes. Here, the cytotoxicity features of an herb- mineral conjugation *Arkeshwara rasa* (AR) has been explored in the primary liver cell line, the Huh7. The same cell lines are seen permissive for the hepatitis viruses and are adopted for simulation studies. (8) The AR includes *Dwigun Kajjali (Parada/* Mercury*: Gandhaka/*Sulphur*=1:2 ratio)* and *Tamra bhasma/*Incinerated copper, in equal proportions. These two main ingredients are later levigated with ***Calotropis procera*** leaf juice, ***Plumbago zeylanica*** root decoction and the three myrobalans (*triphala*) decoction, each 12 times. The previous experimental studies done in AR had checked the cell viability assays on MDA-MB-231 and SW480 cell lines which represented an IC_{50} at 25.28 µg/ml (9) and 40.4 µg/ml, (10) respectively. Similar studies to elicit the anti-cancer activity with AR have been done by Md Nafiujjaman et al on MIA-PaCa-2 and KB cells. (11) Hence to find out the response of

*** Corresponding Author:**
Remya Jayakumar
PhD Scholar, Department of Rasashastra and Bhaishajya Kalpana,
Banaras Hindu University, Varanasi,
UP, India.
Email Id: remyajayakumar@gmail.com

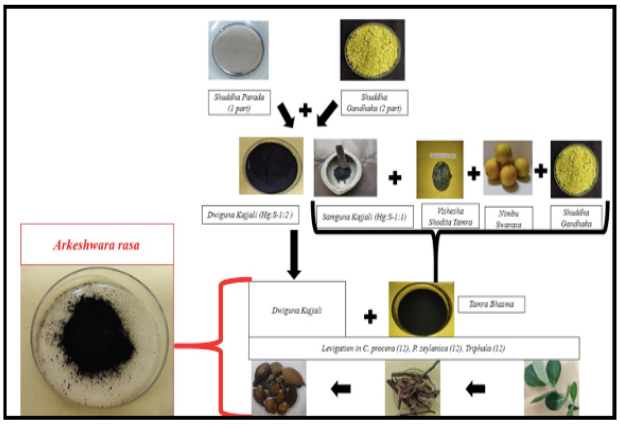
AR on Huh 7 cell lines, this work was propagated. The authors will also intricately explore the possibilities of embedded phytochemicals which have been analyzed in our previous study, (9) to combat HCC.

Material and methods

Processing

The analytical grade Mercury (CAS No:7438-87-6) (12), Sulphur (S094112) (13) and Copper turnings (CAS No:7440-50-8) (14) were subjected to *shodhana* procedures. The *shodith* Mercury and Sulphur were triturated for Kajjali preparation and S*hoditha Tamra* was processed towards *bhasma* preparation (15) and *Amritikaran* (16). This *Tamra bhasma* and *Dwigun Kajjali* were taken in equal proportion for the processing of AR in a modified version. These were levigated with *Calotropis procera* leaf juice, *Plumbago zeylanica* root decoction and *Triphala* decoction, each 12 times. (17) The herbal drugs were authenticated from NISCAIR (National Institute of Science Communication and Information Resources, Council of Scientific and Industrial Research, Ministry of Science and Technology, Government of India). The diagrammatical representation of AR has been done in the **Fig. 1**. The final AR was sent for sophisticated analyzes in different laboratories for characterization. This fine black powder was weighed and stored in air tight containers.

Fig.1: Diagrammatical representation of processing of *Arkeshwara rasa* (AR)



MTT ((3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) Assay of AR against Huh7 cell line

Huh 7 cells were purchased from N.C.C.S, Pune, India and the experimentation was carried at PBRI, Bhopal, India. The cells were treated with DMEM-high glucose media –(Cat no: AL149, Himedia) supplemented with 10 % FBS (#RM10432, Himedia) along with the 1% antibiotic-antimycotic solution in the atmosphere of 5% CO_2, 18-20% O_2 at 37^{0}C temperature in the CO_2 incubator. The 200µl cell suspension in 96-well plate was incubated by adding with the teat agent for 24 hrs. at 37°C in a 5% CO_2 atmosphere. The MTT dye (0.5mg/mL- #4060, Himedia) added plates were again incubated for 3 h at 37 °C in CO_2 incubator. These get used up by the viable cells transforming the yellow tetrazolium salt into purple blue formazan crystals. The formazan crystals after removing the medium were dissolved in dimethyl sulfoxide (DMSO-#PHR1309, Sigma) with incubation for 10 minutes and OD (Optical Density) at 540 nm measured with synergy H1 hybrid microplate reader. Accordingly, the dose–response graph was plotted between concentration of the drug and the inhibition rate. Cell viability percentage was calculated using the formula which forms the ratio between the Absorbance of Sample and Absorbance of Untreated cells × 100 (18).

Ethidium Bromide/Acridine Orange (EtBr/AO) assay of AR with Huh7 cell line

The EtBr/AO Assay is the colorimetric assay to define the apoptosis based on the reaction to Ethidium bromide (50µg/mL solution, Thermo Fischer, USA) and the Acridine orange (20µg/mL solution, Thermo Fischer, USA) on the Huh 7 cell line procured from the NCCS, Pune, India. The cells were cultured in the 12 well plates (Biolite-Thermo) in the concentration of the 2 ×105 cells/ 2 ml and used the cover slips with Poly L-ornithine solution (0.01% -#A-004-M, Sigma), followed by incubation for 24 hours. The cell culture medium used was DMEM- High Glucose media-(Cat No: 2120785, Gibco). The cells were incubated for another 24 hours with test and standard drug (Doxorubicin-#D1515, Sigma) and followed with D-PBS (#TL006, Himedia) wash. The well plates were again washed with D-PBS after removing the cover slips and stained with 200µl solution. These were again washed, mounted on the fluorescence microscope and emission checked with filter cube of Excitation 560/40 nm, Emission with 645/75 nm for EtBr. The Excitation of 470/40 nm and Emission of 525/50 nm for Acridine orange were used for evaluation results (19).

Results

The colorimetric MTT assay provided the half minimal inhibition of the cells at 37.14 µg/ml drug concentration. A decreasing linear pattern of cell viability were also observed with the results. The positive control cells attained the same in a concentration of 5ug/ml of standard Drug Doxorubicin. The formazan crystals and the crenate non-viable cells were identified in close observation. The AO assay in comparison with the control with the IC_{50} drug concentration exhibited the apoptosis features. The cytotoxicity of Doxorubicin standard was not achieved, although the early and late apoptotic features were clearly visible on the sample images. The images of the MTT Assay at different concentrations are expressed in the **Fig no: 2**. The MTT assay values and the respective cell viability are noted in the graphical pattern in **Fig no: 3**. The AO estimated cell line images are represented in the **Fig no: 4**. The **Table no: 1 & 2** represents the validated previous studies on the anti-cancer aspect in various cell lines with the ingredients of AR. This also includes the phytochemicals evaluated as per our previous LC/MS results on different aspects of liver pathogenesis.

ISSN No: 0976-5921
Published online at https://ijam.co.in
International Journal of Ayurvedic Medicine, Supplement of International Conference on Ayurveda-Yoga-Nathpanth - 2025

Figure 2: Representing the different concentration wise MTT assay with AR on Huh 7 cell line

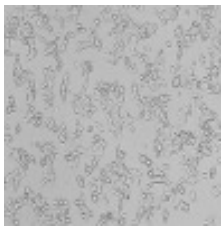
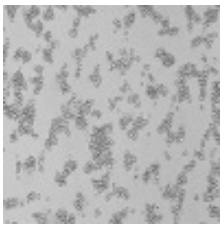
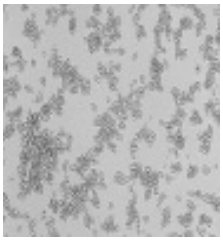
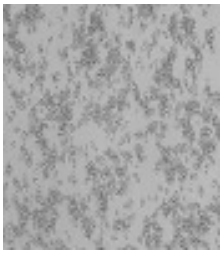
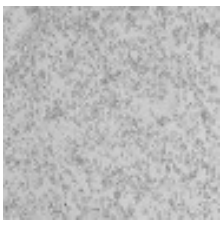
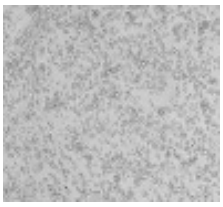
Untreated	STD	100µg/ml	50µg/ml	12µg/ml	6µg/ml
					

Figure 3: Different concentration mediated % of Cell viability of AR treated with Huh7 cells

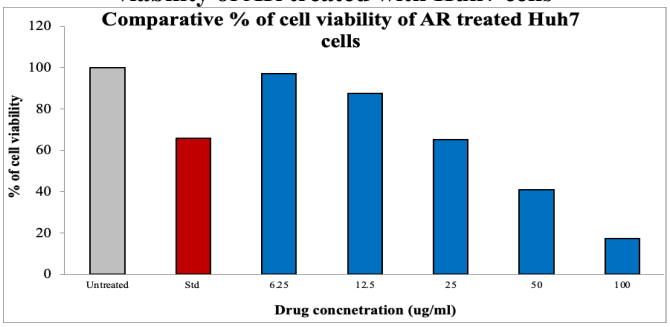


Fig no: 4 Acridine Orange assay with AR representing the VC- volatile cells, EA- Early apoptosis, LA- Late apoptosis in comparison with Untreated and Standard.

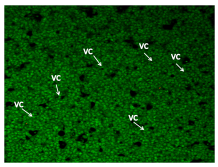
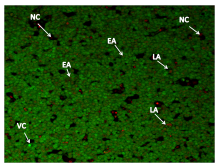
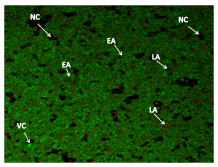
Untreated	STD	Huh 7 cell line with AR
		

Table 2: Hepatic cancer experimental studies with individual *Arkeshwara rasa* (AR) inclusions

AR inclusions	Experimental models	Experimental study details	Observed Results
1. *C. procera* ethanolic leaf extract (20)	Albino rats	Hepatitis B and Acetaminophen induced experiment for hepato-protective activity.	The increase levels of ALT, ALP and AST were brought to normal levels in the treated groups.
2. *Plumbago zeylanica* hexane fraction (21)	EAC cell –Swiss albino mice model	Flow cytometry and cyto toxicity assays	79.01% inhibition of EAC cells at 300mg/Kg. G_0/G1 phase arrest and the BCl_2 downstreaming.
3. *Plumbagin*(22)	HCC, Huh7, HepG2 cell lines	Genotoxicity, oxidative stress, Cell cycle analysis	G2/M phase arrest, γ-H2AX pertaining to DNA damage was increased, suppression p53 and
4. *Plumbago zeylanica* ethanolic root extract (23)	HeLa cancer cell line	MTT assay, wound healing activity, Cell cycle analysis	IC50-10.49 µg/ml, S phase cell cycle arrest
5. *Triphala* with diet (24)	Swiss albino mice	Benzo(a)pyrene induced forestomach papillomagenesis in	Reduced tumour incidences
6. *Triphala* extract (25)	HCC, HepG2, Hep3B	Cytotoxicity, Annexin V, Western blotting	Down regulated anti-apoptotic proteins and increased poly-ADP ribose polymerase cleavage

Table 3: Phytochemicals of AR in different hepatic carcinoma

AR – LC/MS - phytochemicals	Experimental models	Experimental study details	Observed results
1. Quercetin (26)	HCC cell line LM3 and nude mice tumor model	CCK8 assay, Cell cycle, colony formation, Transwell invasion assays	PCNA reduction ad Bax expression. S and G2/M phase
2. Hesperidine (27)	HepG2 cell line	Cell viability, Western blotting, electrophoresis	Absence of caspase activation and PARP cleavage, DNA fragmentation, paraptosis like cell death, activation of ERK1/2 pathway
3. Rutin(28)	HepG2 cells	Cell viability, flow cyto metry, Wound healing assay, Matrigel invasion assay	*IC*50 value of 52.7 µmol L–1, decrease CYP enzyme
4. Kaempferol (29)	Huh-7, Huh-1, HepG2, HepG2.2.15, SK-Hep-1, PLC/PRF/5, HLE, HLF, and Hep3B	Cell viability, cell cycle, mitochondrial apoptosis, wound healing , Western blotting, comparison with Doxorubicin(DOX)	Synergistic with DOX, metalloprotienases and AkT/mTOR down regulation

Discussion

AR was found to be in the tetrahedrite morphology with Copper, Sulphur, Antimony in the XRD peaks and EDAX levels coinciding with Fe, Mg, etc. (9) The FTIR data support the presence of O-H, N-H, S=O stretch vibrations and the finger print region were dominating with phenol (-OH) groups in the graph. (10) So the potential anticancer effects depend on the aggregate synergism of all the components of the AR. The derangements in copper metabolism can support the genesis of HCC and the role of copper cannot be avoided in the normal physiology being a trace element. The liver is also the storage hub of metabolized copper elements. Here, AR includes the *Tamra bhasma*/incinerated copper particles with herbal levigations which exhibits hepato-protective activity. A similar compound known as *Arogyavardhini vati* which also contains *Tamra bhasma* was found to be protective against CCl_4 induced liver toxicity. (30) *Roudra rasa* , a preparation experimented against different cell lines possess anti-cancer activity. (31) Similarly, AR was also found to be reversing the induced changes like vacuolations in the acute and sub-acute toxicity studies on Charles foster female rats. This toxicity study also pointed out the peak of hemoglobin (Hb) concentrations in treated groups than the control and the increase in concentration with escalated dose relates the proper copper metabolism for the maintenance of Hb. The similar rise of Packed Cell Volume (PCV) also coincides with Hb rise pointing out the fact that there was no hemolysis to substantiate the peak Hb level.

The ethanolic leaf extract of ***Calotropis procera*** when tested on HCT 116 and MCF-7 was found to cause apoptosis with cell cycle arrest at G2M phase and Sub-G1 phase respectively. There was loss of cell membrane asymmetry in MCF 7 (breast cancer) and HCT-116 (Colon cancer) cell lines when analyzed with Annexin V-FITC/PI. The same study also points out the dysregulation of cell death markers like Survivin, mTOR, p-mTOR, AKT, p-AKT and the inhibition of the cell cycle regulating proteins like CDK1, CDK4, CDK6 and cyclin B1. The alteration of mTOR and AKT levels suggest the disturbances of cellular proliferations (32) and the Cyclin Dependent Kinase inhibition are related to G1 phase arrest particularly in HCC cells. (33) In the PC-3 cells (prostate cancer), leaf extract was empowering the autophagy procedure which caused the upregulation of *p62, LC3B and Beclin-1* transcript proteins, which support the pre-apoptotic functions. (34) Whereas, the fractionated part of plant was found to exhibit anti-angiogenesis effect on HepG2 (Liver carcinoma) cell line. (35)

The ***Plumbago zeylanica***, root decoction was found to provide hepato-protective effect in paracetamol induced liver failure. (36) The plumbagin, the natural naphthoquinone content in the root extract can generate ROS species. This can produce double strand breaks in DNA triple negative breast cancer. In HepG2 cells, the p53 activation led to anti-proliferative effect. (23) The hepatocyte burden of lipids can be scraped out by Plumbago zeylanica extract. In the KEGG analysis network, the feed back of the plumbagin was found to influence the HCC via cell survival, proliferation, apoptosis, and angiogenesis hindrances. The cross talks between the cells were more focused on the *PI3K-Akt, mTOR* and *MAPK* signaling pathways. (37) This was similar to the Calotropis procera constituents as discussed above.

The inclusions of ***Terminalia chebula*** (38), ***Terminalia bellerica*** (39), ***Emblica officinalis*** (39) exerts hepato-protective activity in various methodologies. The chebulanic acid was found to preserve the hepatic enzymes against the CCl_4 toxicity. (40) The tannins in ***Terminalia bellerica*** can influence the immunomodulatory function with support to CD8+ T cell activities. (41) The methanolic extract of the fruit of ***Emblica officinalis*** was found to resist the carcinogenic activity against DEN (Diethyl nitrosoamine) and AAF (Acetylaminoflourine) induced tumour model in rats. (42) The combined effect was alone found to influence the caspases activation and downstreams the anti-apoptotic genes on pancreatic cell lines. (43)

The asymptomatic progression and advanced stage diagnosis of HCC might be the setback of the rhythmical orchestration of cross talks between different signal pathways. The expression of Galectin 3, the galactoside binding glycoprotein,(44) is found to be directed towards the immunosuppression by binding on to the tumor cells. This supports the maintenance of the TME (Tumor Micro Environment) by propagating the glycolysis and mitochondrial functions. Kaempferol (45) and Quercetin(46) was found to decrease this galactin 3 activating the ligand shield as to escape the receptors of natural killer cells. The same in AR might be presenting the tumor cells in surveillance of apoptosis mechanism as seen in our cytotoxicity assay. The progressive point of JAK/STAT 3 pathway is related to Gut dysbiosis in HBV (Hepatitis B Virus) and develop the HCC later. The hesperidine, neohesperidine, Rutin, Kaempferol, Resveratrol and Luteolin were found to produce the anti-proliferative action via this pathway(47) Moreover, the compounds isolated from the leaf extract of ***Calotropis procera*** like Ferulic acid are also present in the AR chromatographic expression because there was 12 times wet levigation with expressed leaf juice of the plant. (32) This Ferulic acid has been found to reverse the practically impossible fibrotic hepatic changes and the related oxidative stress in a mouse model. The ECM (Extracellular Matrix) rearrangement and deactivation of the HSC (Hepatic stellar cellular) functions are both connected towards the hepatocyte fibrosis. The fibrosis also happens due to the abundant ECM (Extracellular Matrix) deposition and the consequent scaring from diverse conditions including the viral infection to alcohol abuse, steatosis, oxidative stress, etc. Shortly, these fibrotic changes are repeated expression of inflammation and free radical internal milieu. (48)The reversible damage of fibrotic hepatocytes are seen vested along with the downstream transcription of NfkB proteins influenced with Ferulic acid. The further examination with Apoptosis Assay of AR might be producing the similar results due to the above mentioned phyto-constituents.

Immunotherapy has been found beneficial in improving the survival rate from 18% to 43% in recent

studies. The activations of macrophages and the production of NO from them has been seen with *Calotropis* water soluble latex extract. (49) Plumbagin was expressing the immunomodulatory functions by inhibiting the T-cell proliferation with cell cycle blockage as well as supported the viability of the lymphocytes. The NFkB activation was also inhibited by Plumbagin in the same study. (50) Whereas, Triphala manipulates the expression of different cytokines like IL4, IL2, IFN-γ by up and down regulations according to the oxidative stress.(51) The phenolic compounds in AR might also be able to protract the anti-microbial action due to the phenolic proportion with –OH(hydroxyl) groups ensuring the affinity towards the microbes. (52)

α-amylase, the hydrolyser of glycogen is secreted by salivary glands, pancreas and liver which floods out the glucose source to highly proliferated cells. Therefore, the inhibition of the same is well appreciated in both diabetic and Warburg effect prevailing in the cancer cells. Such an inhibition tendency is seen among ethanolic and aqueous extract of *Calotropis procera* leaf. (53) A conjugated presence of copper nano particles with *Plumbago zeylanica* processing were also revealing the same inhibitory activity. (54) As the obesity induced fatty liver changes also express a peak in alpha amylase activity, the same inhibitory potentials were elicited with *Triphala* and its constituents. (55) This effect might be also exhausting the hepatic cancer cells when α-glucosidase is inhibited from progressing against pathogenesis.

The advantage of precision medicine has been found beneficial in many recent works. They carry the essence of targeted delivery. Antimony encapsulated nano-spheres can form such carriers and so the presence of the same in AR might be precisely supporting the targeted delivery of herb- mineral conjugations. Also, the organo-sulphur compounds like GSH (Glutathione), ALA (Alpha Lipoic Acid), Allicin, etc were seen to reduce the damage associated with CCl_4 induced hepatic fibrosis and AR's EDAX point out the presence of Sulphur peaks in the compound.(56)

Ayurveda describes *yakrit*/liver as an organ and one of the *mula sthan* of *raktavaha srotas*. All the treatment aspects of *yakrit* are same as *pleeha*/spleen diseases. These factors also imply the disease management to be focused on the core immune related factors as CD4 cells, T cells, NK cells, etc. The etiological records point out the cause of gut dysbiosis as the consumption of too much salty, sourly, spicy foods which are known to cause *vidahi* (substances that produce burning sensations) and *abhishyanda* (*srothas* obstructing dravya) effect. The described formulations for *yakrit-pleeha vikara* like *Arka lavana, Chitrak grita*, etc constitute the AR ingredients.

Conclusion

AR in the Huh 7 cell line exhibited cytotoxicity and apoptotic features which need to be further examined with both in-vitro and in-vivo experiments. The previous studies and the LC/MS derived metabolites point out the specificity on downregulation of mTOR, NFkB protiens, levelling down of Galactosin, inhibition of amylase and glucosidase, supporting the CD8+ T cells, initiating the autophagy of macrophages, etc which all need to be further experimented. The tendency to infiltrate the neutrophils and maintain the intact copper metabolism has seen in the AR toxicity studies. These all suggest the presence of an ideal potency to combat the HCC related pathogenesis which needs to be confirmed with gene amplification, reverse transcription, cell cycle analysis, Western Blotting, etc.

Abbreviations

- HCC- Hepatocellular Carcinoma
- AR- *Arkeshwara Rasa*
- MTT Assay- (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) Assay
- EtBr/AO -Ethidium Bromide/Acridine orange Assay
- IC_{50}- Half maximal Inhibition Constant
- EAC cell- Ehrlich Ascites Carcinoma cell
- LC/MS- Liquid Chromatography Mass Spectrometry
- ECM – Extracellular Matrix
- NO- Nitric oxide

Acknowledgements

The authors are thankful towards Dr. Megha Jha, Pinnacle Biomedical Research Centre, Contract Work Department, Bhopal, India- for MTT and AO Assay.

References

1. ICD10Data.com. ICD-10 [Internet]. ICD10Data.com. 2024. p. 1. Available from: https://www.icd10data.com/ICD10CM/Codes/C00-D49/C15-C26/C22-/C22.0
2. Alberts CJ, Clifford GM, Georges D, Negro F, Lesi OA, Hutin YJF, et al. Worldwide prevalence of hepatitis B virus and hepatitis C virus among patients with cirrhosis at country, region, and global levels: a systematic review. Lancet Gastroenterol Hepatol [Internet]. 2022;7(8):724–35. Available from: http://dx.doi.org/10.1016/S2468-1253(22)00050-4
3. Philips CA, Rajesh S, Nair DC, Ahamed R, Abduljaleel JK, Augustine P. Hepatocellular Carcinoma in 2021: An Exhaustive Update. Cureus. 2021 Nov;13(11):e19274.
4. Kwabena O. Asafo-Agyei ; Hrishikesh Samant . Hepatocellular Carcinoma - StatPearls - NCBI Bookshelf [Internet]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK559177/
5. Kudo M. All Stages of Hepatocellular Carcinoma Patients Benefit from Systemic Therapy Combined with Locoregional Therapy. Liver Cancer. 2023;12(5):395–404.
6. Chidambaranathan-Reghupaty S, Fisher PB, Sarkar D. Hepatocellular carcinoma (HCC): Epidemiology, etiology and molecular classification. Adv Cancer Res. 2021;149:1–61.
7. Guo L, Hu C, Yao M, Han G. Mechanism of sorafenib resistance associated with ferroptosis in HCC. Front Pharmacol. 2023;14(June 2023):1–11.

8. Sainz BJ, TenCate V, Uprichard SL. Three-dimensional Huh7 cell culture system for the study of Hepatitis C virus infection. Virol J. 2009 Jul;6:103.
9. Jayakumar R, Dash MK, Kumar P, Sharma S, Gulati S, Pandey A, et al. Pharmaceutical characterization and exploration of Arkeshwara rasa in MDA-MB-231 cells. J Ayurveda Integr Med. 2024;15(1):100823.
10. Jayakumar R, Dash MK, Gulati S, Pandey A, Trigun SK, Joshi N. Preliminary data on cytotoxicity and functional group assessment of a herb-mineral combination against colorectal carcinoma cell line. J Complement Integr Med. 2024 Mar;21(1):61–70.
11. Nafiujjaman M, Nurunnabi M, Saha SK, Jahan R, Lee Y-K, Rahmatullah M. Anticancer activity of Arkeshwara Rasa - A herbo-metallic preparation. Ayu. 2015;36(3):346–50.
12. Pranacharya Shri Sadananda Sharma AHS. Rasatarangini. Kashinat P, Shastry H, editors. Motilal Banarasidas, New Delhi; 2014. 5th Taranga, 79–80.
13. Pranacharya Shri Sadananda Sharma AHS. Rasatarangini. Shasthri PK, editor. Motilal Banarasidas, Bungalow Road, New Delhi; 2014. 8th Taaranga, 177-178 pages.
14. Shri Vaidyapati Simha Gupta Suni Vagbata PDAK. Rasaratna Samucchaya- Vijnanabodhini Teeka. Meharchand Lachmandas Publications, New Delhi; 5th Adhyay, Pg no: 93-94.
15. Kulkarni DA. Shri Vaidyapathi Simha Guptha Sunu Vaghbata Acharya, Vijnana Bodhini Teeka. Meharchand Lachmandas Publications, New Delhi; 2017. 5th Adhyaya, /53. Pg no: 101.
16. Sharma PSS. Rasatarangini. Motilal Banarasidas, Bungalow Road, New Delhi; 2014. 17th Taranga/ 37-39, Pg no: 417.
17. Simhaguptha Sunu Vaghbhata PSNM. Simhagupta Sunu Vagbata. Chaukhama orientalia, Varanasi; 2011. 21st Adhyaya, Pg no:490.
18. Ghasemi M, Turnbull T, Sebastian S, Kempson I. The MTT Assay: Utility, Limitations, Pitfalls, and Interpretation in Bulk and Single-Cell Analysis. Int J Mol Sci. 2021 Nov;22(23).
19. Byvaltsev VA, Bardonova LA, Onaka NR, Polkin RA, Ochkal S V, Shepelev V V, et al. Acridine Orange: A Review of Novel Applications for Surgical Cancer Imaging and Therapy. Front Oncol. 2019;9:925.
20. Olaleye M, Akindahunsi A, Ewemade E. Extract Of Calotropis Procera Against Acetaminophen- And Hepatitis Virus- Induced Liver Damage In Rats. Niger J Nat Prod Med. 2008;12(Vol. 12 (2008)).
21. Sharma N, Thakur S, Kour R, Deepika, Kumar A, Ahmad A, et al. Plumbago zeylanica L. exhibited potent anticancer activity in Ehrlich ascites carcinoma bearing Swiss albino mice. J King Saud Univ - Sci [Internet]. 2023;35(9):102932. Available from: https://www.sciencedirect.com/science/article/pii/S1018364723003944.
22. Liu H, Zhang W, Jin L, Liu S, Liang L, Wei Y. Plumbagin Exhibits Genotoxicity and Induces G2/M Cell Cycle Arrest via ROS-Mediated Oxidative Stress and Activation of ATM-p53 Signaling Pathway in Hepatocellular Cells. Int J Mol Sci. 2023;24(7).
23. Mohapatra S, Mohanty J, Pani S, Hansdah S, Biswal AK, Sahoo AK, et al. Root extract of Plumbago zeylanica L. induces cytotoxicity, inhibits cell migration and induces S-phase cell cycle arrest through down regulation of EGFR in HeLa cervical cancer cells. Adv Cancer Biol - Metastasis [Internet]. 2022;4:100027. Available from: https://www.sciencedirect.com/science/article/pii/S2667394022000016
24. Deep G, Dhiman M, Rao AR, Kale RK. Chemopreventive potential of Triphala (a composite Indian drug) on benzo(a)pyrene induced forestomach tumorigenesis in murine tumor model system. J Exp Clin Cancer Res. 2005 Dec;24(4):555–63.
25. Mishra Y, Yadav RR, Verma K, Yadav N. Triphala extract Reduces Anti-apoptotic Protein (Mcl-1) Expression through the Activation of Caspase 3 in Hepatocellular Carcinoma Cells. J Appl Biol Biotechnol. 2024;12(3):176–84.
26. Wu L, Li J, Liu T, Li S, Feng J, Yu Q, et al. Quercetin shows anti-tumor effect in hepatocellular carcinoma LM3 cells by abrogating JAK2/STAT3 signaling pathway. Cancer Med. 2019 Aug;8(10):4806–20.
27. Yumnam S, Park HS, Kim MK, Nagappan A, Hong GE, Lee HJ, et al. Hesperidin induces paraptosis like cell death in hepatoblatoma, Hepg2 cells: Involvement of ERK1/2 MAPK. PLoS One. 2014;9(6).
28. Karakurt S. Modulatory effects of rutin on the expression of cytochrome P450s and antioxidant enzymes in human hepatoma cells. Acta Pharm. 2016;66(4):491–502.
29. Yang G, Xing J, Aikemu B, Sun J, Zheng M. Kaempferol exhibits a synergistic effect with doxorubicin to inhibit proliferation, migration, and invasion of liver cancer. Oncol Rep. 2021 Apr;45(4).
30. Jamadagni S, Jamadagni P, Angom B, Mondal D, Upadhyay S, Gaidhani S, et al. Tissue distribution of mercury and copper after Aarogyavardhini Vati treatment in rat model of CCl(4) induced chronic hepatotoxicity. J Ayurveda Integr Med. 2020;11(4):508–14.
31. Chaurasia SN, Singh V, Ekhlak M, Dash MK, Joshi N, Dash D. Ayurvedic preparations of Raudra Rasa inhibit agonist-mediated platelet activation and restrict thrombogenicity without affecting cell viability. FEBS Open Bio. 2023 Dec;13(12):2342–55.
32. Bou Malhab LJ, Bajbouj K, Shehab NG, Elayoty SM, Sinoj J, Adra S, et al. Potential anticancer properties of calotropis procera: An investigation on breast and colon cancer cells. Heliyon. 2023 Jun;9(6):e16706.
33. Shen S, Dean DC, Yu Z, Duan Z. Role of cyclin-dependent kinases (CDKs) in hepatocellular carcinoma: Therapeutic potential of targeting the CDK signaling pathway. Hepatol Res. 2019;49(10):1097–108.
34. Singh P, Dhole B, Choudhury J, Tuli A, Pandey D, Velpandian T, et al. Calotropis procera extract inhibits prostate cancer through regulation of autophagy. J Cell Mol Med. 2024;28(6):1–16.
35. Khan MF, Alanazi RF, Baabbad AA, Almoutiri ND, Wadaan MA. Angiogenic protein profiling, phytochemical screening and in silico anti-cancer

targets validation of stem, leaves, fruit, and seeds of Calotropis procera in human liver and breast cancer cell lines. Environ Res [Internet]. 2024;256:119180. Available from: https://www.sciencedirect.com/science/article/pii/S0013935124010855
36. Akhilraj AR, Bhat S, Priyalatha B, Vimala KS. Comparative hepatoprotective activity of detoxified roots of Plumbago zeylanica L. and Plumbago rosea L. in Wistar rats. J Ayurveda Integr Med [Internet]. 2021;12(3):452–7. Available from: https://doi.org/10.1016/j.jaim.2021.04.002
37. Li T, Lv M, Chen X, Yu Y, Zang G, Tang Z. Plumbagin inhibits proliferation and induces apoptosis of hepatocellular carcinoma by downregulating the expression of SIVA. Drug Des Devel Ther. 2019;13:1289–300.
38. Choi M-K, Kim H-G, Han J-M, Lee J-S, Lee JS, Chung SH, et al. Hepatoprotective Effect of Terminalia chebula against t-BHP-Induced Acute Liver Injury in C57/BL6 Mice. Evid Based Complement Alternat Med. 2015;2015:517350.
39. Gupta A, Kumar R, Ganguly R, Singh AK, Rana HK, Pandey AK. Antioxidant, anti-inflammatory and hepatoprotective activities of Terminalia bellirica and its bioactive component ellagic acid against diclofenac induced oxidative stress and hepatotoxicity. Toxicol Reports. 2021;8(January 2020):44–52.
40. Feng X-H, Xu H-Y, Wang J-Y, Duan S, Wang Y-C, Ma C-M. In vivo hepatoprotective activity and the underlying mechanism of chebulinic acid from Terminalia chebula fruit. Phytomedicine [Internet]. 2021;83:153479. Available from: https://www.sciencedirect.com/science/article/pii/S0944711321000210
41. Chang Z, Zhang Q, Hu Q, Liu Y, Zhang L, Liu R. Tannins in Terminalia bellirica inhibits hepatocellular carcinoma growth via re-educating tumor-associated macrophages and restoring CD8+T cell function. Biomed Pharmacother [Internet]. 2022;154:113543. Available from: https://www.sciencedirect.com/science/article/pii/S0753332222009325
42. Sultana S, Ahmed S, Jahangir T. Emblica officinalis and hepatocarcinogenesis: A chemopreventive study in Wistar rats. J Ethnopharmacol [Internet]. 2008;118(1):1–6. Available from: https://www.sciencedirect.com/science/article/pii/S0378874108001827
43. Prasad S, Srivastava SK. Oxidative Stress and Cancer: Chemopreventive and Therapeutic Role of Triphala. Antioxidants (Basel, Switzerland). 2020 Jan;9(1).
44. Guo Y, Shen R, Yu L, Zheng X, Cui R, Song Y, et al. Roles of galectin-3 in the tumor microenvironment and tumor metabolism (Review). Oncol Rep. 2020;44(5):1799–809.
45. Chae JB, Choi HR, Shin JW, Na JI, Huh CH, Park KC. The effects of the 3-OH group of Kaempferol on interfollicular epidermal stem cell fate. Ann Dermatol. 2018;30(6):694–700.
46. Li H, Xiao L, He H, Zeng H, Liu J, Jiang C, et al. Quercetin Attenuates Atherosclerotic Inflammation by Inhibiting Galectin-3-NLRP3 Signaling Pathway. Mol Nutr \& Food Res [Internet]. 2021;65(15):2000746. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/mnfr.202000746
47. Yin Q, Wang L, Yu H, Chen D, Zhu W, Sun C. Pharmacological Effects of Polyphenol Phytochemicals on the JAK-STAT Signaling Pathway. Front Pharmacol. 2021;12(September):1–18.
48. Wu J, Xue X, Fan G, Gu Y, Zhou F, Zheng Q, et al. Ferulic Acid Ameliorates Hepatic Inflammation and Fibrotic Liver Injury by Inhibiting PTP1B Activity and Subsequent Promoting AMPK Phosphorylation. Front Pharmacol. 2021;12(September):1–16.
49. Seddek A latif S, Mahmoud ME, Shiina T, Hirayama H, Iwami M, Miyazawa S, et al. Extract from Calotropis procera latex activates murine macrophages. J Nat Med. 2009 Jul;63(3):297–303.
50. Checker R, Sharma D, Sandur SK, Khanam S, Poduval TB. Anti-inflammatory effects of plumbagin are mediated by inhibition of NF-kappaB activation in lymphocytes. Int Immunopharmacol. 2009 Jul;9(7–8):949–58.
51. Belapurkar P, Goyal P, Tiwari-Barua P. Immunomodulatory effects of triphala and its individual constituents: a review. Indian J Pharm Sci. 2014;76(6):467–75.
52. Ahmad Nejhad A, Alizadeh Behbahani B, Hojjati M, Vasiee A, Mehrnia MA. Identification of phytochemical, antioxidant, anticancer and antimicrobial potential of Calotropis procera leaf aqueous extract. Sci Rep [Internet]. 2023;13(1):1–13. Available from: https://doi.org/10.1038/s41598-023-42086-1
53. Kazeem MI, Mayaki AM, Ogungbe BF, Ojekale AB. In-vitro Studies on Calotropis procera Leaf Extracts as Inhibitors of Key Enzymes Linked to Diabetes Mellitus. Iran J Pharm Res IJPR. 2016;15(Suppl):37–44.
54. Jamdade DA, Rajpali D, Joshi KA, Kitture R, Kulkarni AS, Shinde VS, et al. Gnidia glauca- and Plumbago zeylanica-Mediated Synthesis of Novel Copper Nanoparticles as Promising Antidiabetic Agents. Adv Pharmacol Sci. 2019;2019:9080279.
55. Peterson CT, Denniston K, Chopra D. Therapeutic Uses of Triphala in Ayurvedic Medicine. J Altern Complement Med. 2017 Aug;23(8):607–14.
56. Milito A, Brancaccio M, D'Argenio G, Castellano I. Natural sulfur-containing compounds: An alternative therapeutic strategy against liver fibrosis. Cells. 2019;8(11):1–19.

*****