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ANTI-PROLIFERATIVE EFFECT OF *PATHYADI KVATHA* ON HEPATOCELLULAR CARCINOMA

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ABSTRACT

Background:

Ayurveda, the oldest medical system, focused on preventing diseases and maintaining a healthy body. Nowadays cancer is a global concern. In *Ayurveda*, cancer treatment can be correlated with *arbuda, apachi, granthi roga*, etc. A prospective study is being conducted to evaluate the effect of *Pathyadi kvatha* on Hepatocellular carcinoma classically which is mentioned for *yakrita* (liver) and *pleeha* (spleen) *roga* (disease).

Material and methods:

The *Pathyadi kvatha* formulation extract was prepared in aqueous and Hydro-alcoholic media. Classically, the ratio of ingredients for *kvatha* is not mentioned in *shloka* (verse). So, the general ratio mentioned in *Sharangdhara Samhita* was followed. The ratio of ingredients that can

be used for *kvatha* preparation was followed for extract preparation and tested for antiproliferative activity by MTT Assay.

Results:

Pathyadi Kvatha's water-soluble extract showed to be more effective than the Hydro-alcoholic extract. The Hydro-alcoholic extract of the standard drug Cisplatin was substantially less efficient in each interval than the aqueous extract of Cisplatin. When data of water-soluble extract of *Pathyadi kvatha* was compared with water soluble extract of Cisplatin it was found that Cisplatin was better than *Pathyadi kvatha*.

Conclusion:

In the current study, extracts of medications specified in the *Pathyadi Kvatha* formulation were employed in the same ratio as the kvatha preparation. Individually, extracts of these drugs have shown anti-cancerous activity in various research models. Further, in vivo and clinical research is needed to understand the formulation's effects completely.

Keywords: *Pathyadi kvatha*, Cancer, Hepatocellular carcinoma, *Ayurveda*

Abbreviations:

HBV	**Hepatitis B**
HCC	**Hepatocellular carcinoma**
HCl	**Hydrochloric Acid**
HCV	**Hepatitis C**
HepG2	**Hepatoblastoma**
Mg	**Milligram**
µg	**Microgram**

1. INTRODUCTION

Ayurveda is the science of life and the oldest medical system. *Ayurveda* is regarded as the *upaveda* of *Atharvaveda* **[1]**. The goal of *Ayurveda* is not only to prevent diseases but also to preserve a healthy state of mind in a healthy body.

Nowadays, cancer is a major public health concern. Cancer is a type of illness in which cells proliferate, invade, and occasionally metastasize uncontrollably. In The United States, 1,958,310 new cases of cancer and 609,820 cancer deaths are projected to occur in 2023 **[2]**. Globally, 9.6 million people died from cancer in 2018, according to GLOBOCAN. It is anticipated that there will be 16.3 million cancer deaths and 29.5 million new cases of the disease by 2040 **[3]**.

Hepatocellular carcinoma (primary liver cancer) is the third most prevalent cause

of cancer-related mortality globally and is the fifth most common cancer in men and the seventh most common in women. The primary known risk factors for HCC are chronic HBV or HCV infections. In Africa and eastern Asia, exposure to aflatoxin is a significant risk factor for the development of HCC. Heavy alcohol use, cigarette use, obesity, diabetes, genetic variables, and certain dietary issues all play a role in the development of HCC in high-income nations **[4]**.

Arbuda, *apachi*, and *granthyadi roga* are associated with cancer in *Ayurveda*, and the basis of the recommended treatment has also been covered in this context. *Pathyadi kvatha* is described in *Sharangadhara Samhita* for *yakrita* (liver) and *pliha* (spleen) diseases. This formulation contains mainly two ingredients *Haritaki (Terminalia chebula)* and *Rohitaka (Tecomella undulla)*. There are two *prakshepah dravyas* namely *Pippali* (*Piper longum*) and *Yavakshara* (phyto alkali of *Hordeum vulgare*), which are fine powders added to the decoction **[5]**.

According to previous research, few of the ingredients in this preparation have anti-proliferative properties. *Haritaki* (T. chebula fruit) crude extract and phenolics decrease the number of cells in cancer cell lines by suppressing cell development and increasing cell death **[6]**. *Pippali* (*Piper longum*) also has hepatoprotective properties **[7]**. A prospective study of *Pathyadi kvatha's* anti-proliferative activity on HepG2 cells is undertaken to evaluate its combined effect on hepatocellular carcinoma.

2. MATERIAL AND METHODS

For the present study, ingredients of *Pathyadi kvatha* were procured from two different places. *Pippali*, *Haritaki*, and *Yavakshara* were purchased from K. Govindraja Setty & Sons, Mysuru. Whereas, *Rohitaka* was purchased from Herbal Health Research Consortium Pvt. Ltd., Amritsar. Authentication of drugs was done in the department of Dravyaguna, SDM Ayurvedic Medical College, and Hospital Hassan.

Then the extract was prepared at SDM Ayurvedic College and Hospital, Udupi. Because for MTT Assay extract of drugs is required. The extract was prepared with 5g *Rohitaka (Tecomella undulla)*, 5g *Haritaki (Terminalia chebula)*, 2.4g *Pippali* (*Piper longum*), and 0.6g *Yavakshara* (Phyto alkali of *Hordeum vulgare*). The aqueous and Hydro-alcoholic extracts were prepared and used to assess the antiproliferative activity.

To check the efficacy of *Pathyadi kvatha* its results were compared with standard drug. For the present study, Cisplatin was taken as a standard drug. As needed for the MTT assay protocol, aqueous and hydro-

alcoholic extracts were also taken for the standard group.

2.1 Method of preparation of aqueous and hydro-alcoholic extract

In 100 ml of distilled water, *rohitaka*, *haritaki*, and *pippali's yavakuta churna* were mixed. A cotton ball and parafilm paper were used to seal the flask's mouth, and it was shaken on the spinix orbital shaker for 40 hours and 10 minutes at a speed of 16.1 or 16.2 RPM.

Later, it was filtered using a filter paper. The filtrate obtained was put into a china dish and placed in a hot air oven to dry. The extract was then supplemented with *Yavakshara*. Finally, an aqueous extract of the *Pathyadi kvatha* was obtained.

Similarly, hydro-alcoholic extract was prepared with 50 ml of ethanol and 50 ml of distilled water. The remaining procedure was similar as the aqueous extract.

2.2 Screening of cell viability by MTT assay

2.2.1 Aim:

Screening of Hepatocellular cell viability by MTT assay for *Pathyadi kvatha* and standard drug Cisplatin.

2.2.2 Materials required:

MTT (Sigma Aldrich), Dimethyl sulfoxide (DMSO), Phosphate buffer saline (PBS), 96 well plate

2.2. Procedure:

A confluent cell line flask was taken out, and the cells were trypsinized. The cells were washed with Phosphate Buffer Saline (PBS) twice and centrifuged. The pellet was resuspended in an appropriate medium (medium containing 10% Fetal bovine serum) and the cells were counted by using a hemocytometer. The cells were seeded in 96-well plates (10,000 cells per well) and the plate was incubated at 37º C in a CO_2 incubator for 24 hours. After 24 hours, the old medium was carefully discarded from the 96-well plate. The different concentration of the drug in a suitable serum-free medium was dissolved and added to different test groups. The plate was incubated for 48 hours at 37º C in a CO_2 incubator. After completion of incubation time, 20 µL of MTT dye (5 mg/mL in PBS) was added to all wells. The plate was sealed in aluminum foil and placed in a CO_2 incubator for 4 hours. After 4 hours, 100 µl of acidified Isopropanol was added to each well and mixed carefully by orbital mixing. Using a multiwall plate reader, the absorbance at 540 nm (or 540 nm with reference to 630 nm) was recorded.

2.2.4 Assessment criteria:

The effect of aqueous extract and hydro-alcoholic extract of *Pathyadi kvatha* was assessed based on the percentage of viable cells calculated with the help of MTT assay.

The following formula was used to determine the percentage of viable cells:

% of viable cells = [(Test sample-blank) / (Control-blank)] x 100

2.2.5 Observations

The observations are tabulated in **Table 1**.

2.2.6. RESULTS

For *Pathyadi kvatha's* aqueous extract at 1000 µg/ml concentration, 13.856% of the cells were viable. Whereas in hydro-alcoholic extract 18.005% of the cells were viable. Hence, it was revealed that the aqueous extract of *Pathyadi Kvatha* had more significant and efficient results than the Hydro-alcoholic extract.

Then the results of the aqueous extract of *Pathyadi kvatha* were compared with the standard drug, Cisplatin (aqueous extract). It was noted that at the concentration of 1000µg/ml, 0.594% of cells were viable in the aqueous extract of Cisplatin whereas, in the aqueous extract of *Pathyadi kvatha*, 13.856% of cells were viable. This indicates Cisplatin extract was more significant than the *Pathyadi Kvatha* extract.

3. DISCUSSION

Using the MTT assay to evaluate the anti-cancerous activity of an aqueous and Hydro-alcoholic extract of *Pathyadi kvatha* against that of the well-known anticancer agent Cisplatin, it was discovered that the medicine was most effective at a concentration of 5000 µg/ml. The hydro-alcoholic extract of *Pathyadi kvatha* has 9.051% viable cells at 5000 µg/ml, compared to 8.105% viable cells in the aqueous extract. This suggests that the Hydro-alcoholic extract of *Pathyadi Kvatha* has not performed as well as the aqueous extract. This explains why aqueous media were selected to prepare *kvatha* classically.

For the standard medication Cisplatin, both aqueous and Hydro-alcoholic extracts were tested. At a concentration of 1000 µg/ml, aqueous extracts had better results. 99.406% of the cells at this concentration were dead. The drug Cisplatin was licensed by the US Food and Drug Administration in 1979 and is now most frequently used in chemotherapy to treat various cancers. Although it works better against testicular cancer, Cisplatin demonstrates considerable outcomes against ovarian, bladder, lung, head, and neck tumors. Because of the use of chemotherapy based on Cisplatin, more than 90% of testicular tumors are curable. Other solid tumor's clinical progression is slowed down by Cisplatin, although complete recovery is unusual. In many cancers, drug resistance is frequently the root of treatment failure **[8]**.

Despite the fact that Cisplatin has been available to consumers for more than 40 years,

it still has serious dose-limiting adverse effects, most notably nephrotoxicity, which can be dose-limiting in 30–40% of patients **[9]**. Among the symptoms of Cisplatin nephrotoxicity are acute kidney injury, hypomagnesemia, hypocalcaemia, hyperuricemia, distal renal tubular acidosis, proximal tubular dysfunction, and chronic renal failure. The most severe and frequent manifestation of these is acute renal injury, which affects 20–30% of patients **[10]**.

At a higher concentration of 5000 µg/ml, the aqueous extract seems to provide more significant results for *Pathyadi kvatha* than the Hydro-alcoholic extract. In order to have an anti-proliferative effect, *Pathyadi kvatha* needs to be administered at higher doses. So far, no adverse effects related to *Pathyadi Kvatha* have been reported. Therefore, it can be chosen over Cisplatin, which has a lot of morbid side effects.

There are two main ingredients used in the preparation of *Pathyadi kvatha* i.e., *Haritaki* (*Terminalia chebula*), and *Rohitaka* (*Tecomella undulla*), with *Yavakshara* (Phyto alkali from *Hordeum vulgare*), *Pippali* (*Piper longum*) as *prakshepah*. *Haritaki* and *Pippali* have been the subject of anti-cancer studies in the past, and it has been shown that they have an anti-cancer effect. According to a study on *Haritaki*, the strongest inhibiting phenolics were chebulinic acid, tannic acid, and ellagic acid **[11]**. In an in vivo investigation, *Rohitaka* exhibited hepatoprotective activity **[12]**. Researches done on *Pippali* has also shown that the herb has hepatoprotective properties **[13]**.

4. CONCLUSION

In the form of a decoction (kvatha), the Ayurvedic pharmaceutics of *Bhaishajya Kalpana* outline the extraction of the medicine's heat-resistant components. For the present study, extracts of *Pathyadi Kvatha* drugs were used. *Pippali* (*Piper longum),* mentioned as *prakshepah dravya*, was also added while taking the extract, whereas *Yavakshara* (Phyto alkali of *Hordeum vulgare*) was added after preparation as it is an alkali. Cisplatin showed better results compared to the test drug. Individual components of *Pathyadi Kvatha* have demonstrated anti-cancerous activity in several research models. To fully comprehend the results of this formulation, additional research, such as in vivo and clinical research, must be conducted.

5. Appendix:

Table 1: The percentage (%) of viable cells - HepG2 cell lines treated with aqueous extract and hydro-alcoholic extract of *Pathyadi kvatha*

Concentration of extract in (µg / ml)	Viability percentage (%) of *Pathyadi kvatha* aqueous extract (Mean ±SE)	Viability percentage (%) of *Pathyadi kvatha* Hydro-alcoholic extract (Mean ±SE)
Control	100	100
1	69.392 ± 5.121	83.481 ± 2.869
2	47.215	62.635
4	33.712	55.445
8	25.569	38.804
10	23.786	35.350
20	21.990	33.969
40	21.115	30.924
80	19.888	28.035
100	18.587	27.278
200	17.982	22.318
400	17.292	20.515
800	14.553	18.740
1000	13.856	18.005
2000	11.986	15.093
4000	11.006	11.657
5000	8.105	9.051
Cisplatin 500 (µg / ml)	1.961	2.236
Cisplatin 1000 (µg / ml)	0.594	0.880

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