In Vitro Evaluation of Cytotoxicity and Anticancer Activity of Herbal Extract V2S2 (Coded Drug) on Human Cancer Cell Lines

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Abstract

Background Cancer is the leading cause of morbidity and mortality worldwide. It encompasses a large family of diseases exhibiting abnormal cell growth with the potential to invade or spread to other parts of the body. Natural extracts have been used as an efficient anticancer agent that show promising results. In this study, the cytotoxicity and anticancer effect of Ayurvedic herbal extract code name V2S2 was studied and compared with control drug Adriamycin that is an established chemotherapeutic drug.

Materials and Methods Ayurvedic coded drug V2S2 is a hydroalcoholic extract of herbs. The in vitro anticancer study was performed with sulforhodamine B assay in TATA Memorial, ACTREC Mumbai as per National Cancer Institute guidelines.

Result GI50 of V2S2 study drug and Adriamycin on COLO-205, HOP-62, K-562, ISHIKAWA, HeLa, MCF-7, SCC-40, Hep-G2 and MIA-PA-CA-2 is less than 10 that means both drugs have equal anticancer activity.

Conclusion The study group has more and less equal potential of anticancer activity in compared with a transient’s standard anticancer drug. Moreover, in vitro monkey kidney normal cell line study drug V2S2 shows same cytotoxicity as shown by control drug Adriamycin.

Keywords ► cancer ► GI50 ► V2S2 ► Adriamycin

Introduction

Cancer is the major cause of morbidity and mortality and represents a foremost public health concern worldwide. The phenotype is characterized by abnormal cell growth with the potential to invade or spread to other parts of the body.1 According to the latest World Health Organization International Agency for Research on Cancer Global Cancer Observatory report,2 19.3 million new cases of cancer with 10.0 million death have been reported this year. Indian Statistics-2018 (National Institute of Cancer Prevention and Research) shows 2.25 million peoples are living with the disease, in which 11,57,294 new cancer patients have been registered, while 7,84,821 are cancer-related deaths.3 Chemotherapy, radiotherapy, hormone therapy, surgical treatments, and several anticancer agents are the only current treatment options; however, these approaches are limited due to associated deleterious side effects.4 The world is still struggling for effective, cheap, and safe therapeutic agent. As far as the traditional system of Indian medicine is concerned, herbs are attractive target in cancer therapies owing to their low toxicity and cost. Earlier evidence elucidates natural extracts
as well as phytochemicals derived from plants have the potential of anticancer activity as observed in vincristine\textsuperscript{5} or camptothecin\textsuperscript{6} and proven to be inevitable and valuable resource in cancer therapy. Even vegetables and fruits may help to reduce the risk of cancer in humans.\textsuperscript{7,8}

V2S2, a polyherbal extract, is claimed to have diverse medicinal activities. However, to the best of our knowledge, no study has been conducted to address the potential anticancer activity of V2S2. In this perspective, we have made an attempt to evaluate the anticancer activity of V2S2 and compared its efficacy with the previously reported chemotherapeutic drug Adriamycin.

**Materials and Methods**

**Collection of Plant Materials and Preparation of Extract**

All the plants used in the study were obtained from Indian states of Jammu & Kashmir, Uttarakhand, and Himachal Pradesh and authentication was confirmed by Sharad Porte, Associate Professor, Department of Toxicology, National Institute of Ayurveda (NIA), Jaipur, Rajasthan. After collection, preprocessing was performed in NIA pharmacy. All the raw materials were cleaned, washed, and dried in shade for preservation, restricting fungal growth. The plants’ part was pulverized up to 5 mm mesh size and stored in airtight container. The dried powder of above-mentioned herbs was pulverized and used for extraction with the help of Soxhlet hydroalcoholic extraction method.\textsuperscript{9} Dried herbs (250 g) were extracted in methanol and water (1:1). Extraction was performed in four cycles of 3 hour in a concentration up to 50 total dissolved solid in reactor at 70 to 80°C under reduced pressure. The mixture was dried in vacuum rotatory dryer (Hieroglyph Instruments, Germany) at 80°C. The lumps were removed after cooling to ambient temperature. Multi-milling was done and sieved through #40. The finished product was packed in triple-layer aluminum sealable pouches. About 20% (50 g) extraction was achieved as compared with total quantity of raw material.

**Cell Culture**

Cancer cell lines, COLO-250 (colon), HOP-62 (lung), K-562 (leukemia), ISHIKAWA (endometrium), HeLa (cervical), MCF-7 (breast), Hep-G2 (hepatoma), MIA-PA-CA-2 (pancreas),

![Fig. 1](image-url)  
**Fig. 1** Effect of different concentrations of V2S2 and Adriamycin on human colon cancer cell line Colo-205.
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**Fig. 2** Effect of different concentrations of V2S2 and Adriamycin on human lung cancer cell line HOP-62.

**Fig. 3** Effect of different concentrations of V2S2 and Adriamycin on human leukemia cell line K-562.
### Fig. 4
Effect of different concentrations of V2S2 and Adriamycin on human endometrial adenocarcinoma ISHIKAWA.

### Table 4: Human Endometrial Adenocarcinoma ISHIKAWA

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
<th>Average Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>V2S2</td>
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<td>19.1</td>
<td>19.6</td>
<td>29.9</td>
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<td>32.8</td>
<td>35.6</td>
<td>12.3</td>
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<tr>
<td>ADR</td>
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<td>7.6</td>
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<td>27.5</td>
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</table>

### Graph 4: Growth Curve
- **Drug Concentration (µg/ml)**: 10, 20, 40, 80
- **% Control Growth**
  - V2S2: NE to 100%
  - ADR: NE to 50%

### Table 5: Parameters Calculated from Graph

<table>
<thead>
<tr>
<th>Concentrations (µg/ml)</th>
<th>LC50</th>
<th>TGI</th>
<th>GI50</th>
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<tbody>
<tr>
<td>V2S2</td>
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<td>NE</td>
<td>&lt;10</td>
</tr>
<tr>
<td>ADR</td>
<td>NE</td>
<td>NE</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

**Impression:** The sample V2S2 was active on Human Endometrial Adenocarcinoma ISHIKAWA in the assay system used.

### Fig. 5
Effect of different concentrations of V2S2 and Adriamycin on human cervical cancer cell line HeLa.

### Table 5: Human Cervical Cancer Cell Line HeLa

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
<th>Average Values</th>
</tr>
</thead>
<tbody>
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<td>9.2</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>8.7</td>
<td>21.9</td>
<td>26.2</td>
<td>18.8</td>
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<td></td>
<td>10.5</td>
<td>27.6</td>
<td>26.5</td>
<td>18.2</td>
</tr>
<tr>
<td>ADR</td>
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<td>5.0</td>
<td>2.0</td>
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<td>-21.2</td>
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<td>-5.0</td>
<td>-1.5</td>
</tr>
</tbody>
</table>

### Graph 5: Growth Curve
- **Drug Concentration (µg/ml)**: 10, 20, 40, 80
- **% Control Growth**
  - V2S2: NE to 100%
  - ADR: NE to 50%

### Table 5: Parameters Calculated from Graph

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</tr>
</thead>
<tbody>
<tr>
<td>V2S2</td>
<td>NE</td>
<td>NE</td>
<td>&lt;10</td>
</tr>
<tr>
<td>ADR</td>
<td>NE</td>
<td>NE</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

**Impression:** The sample V2S2 was active on Human Cervical Cancer Cell Line HeLa in the assay system used.
and Vero (kidney), were obtained from TATA ACTREC, Mumbai, Maharashtra. The cells were grown and maintained in appropriate medium at pH 7.4 supplemented with 10% fetal calf serum, glutamine (2mM), penicillin (100 units/mL), and streptomycin (100µg/mL). The cells were grown in CO₂ incubator (Heraeus, GmbH, Germany) at 37°C with 90% humidity and 5% CO₂.

**Anticancer Activity Using Sulforhodamine B Assay**

Cell lines were grown in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% fatal bovine serum and 2 mM L-glutamine. Cells were inoculated into 96 well microtiter plates in 100 µL at plating density, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°C, 5% CO₂, 95% air, and 100% relative humidity for 24 hours prior to addition of experimental drugs. V2S2 was initially solubilized in dimethyl sulfoxide at 100 mg/mL and diluted to 1 mg/mL using water and stored frozen prior to use. Using the six absorbance measurements (time zero [Tz], control growth [C], and test growth in the presence of drug at the four concentration levels [Ti]), the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as \[\frac{Ti}{C} \times 100\%\].

**Result**

Initially, we investigated the inhibitory effect of V2S2 extract on different cancer cell lines. The anticancer activity of study drug was measured and compared with Adriamycin, measuring the percentage growth inhibition of human cell line by using microplate reader. The drug concentration of both study as well as standard control drugs was used as per the guidelines of National Cancer Institute (NCI), like 10 µg/mL, 20 µg/mL, 40 µg/mL, and 80 µg/mL. The present experiment was repeated thrice and then average percentage of control growth was calculated and compared.

The average percentage control growth of V2S2 on human colon cancer cell line (as shown in Fig. 1) (COLO-250) is −33.3 on 10 µg/mL, −20.3 on 20 µg/mL, −10.1 on 40 µg/mL, −10.7 on 80 µg/mL, whereas average percentage control growth of Adriamycin on COLO-250 is −34.9 on 10 µg/mL, −27.5 on 20 µg/mL, −25.1 on 40 µg/mL, −40.1 on 80 µg/mL.

![Fig. 6 Effect of different concentrations of V2S2 and Adriamycin on human cancer cell line MCF-7.](image)
As shown in ►Fig. 2, the average percentage control growth of V2S2 on human lung cancer cell line (HOP-62) is 2.6 on 10 µg/mL, 20.0 on 20 µg/mL, 22.2 on 40 µg/mL, 1.3 on 80 µg/mL, while average percentage control growth of Adriamycin on HOP-62 is 2.5 on 10 µg/mL, 17.7 on 20 µg/mL, −17.6 on 40 µg/mL, −32.4 on 80 µg/mL. Similarly, impact of the average percentage control growth of V2S2 on human leukemia cell (K-562) is 34.6 on 10 µg/mL, 30.7 on 20 µg/mL, 22.2 on 40 µg/mL, 27.7 on 80 µg/mL, whereas average percentage control growth of Adriamycin on K-562 is 38.4 on 10 µg/mL, 17.6 on 20 µg/mL, −6.8 on 40 µg/mL, 10.1 on 80 µg/mL (►Fig. 3).

The average percentage control growth of V2S2 on human endometrial adenocarcinoma (ISHIKAWA) is 29.5 on 10 µg/mL, 24.4 on 20 µg/mL, 27.5 on 40 µg/mL, 8.2 on 80 µg/mL, while average percentage control growth of Adriamycin on ISHIKAWA is 19.4 on 10 µg/mL, 32.1 on 20 µg/mL, 26.5 on 40 µg/mL, −17.2 on 80 µg/mL (►Fig. 4). As shown in ►Fig. 5, the average percentage control growth of V2S2 on human cervical cancer cell line (HeLa) is 9.1 on 10 µg/mL, 19.1 on 20 µg/mL, 20.6 on 40 µg/mL, 15.1 on 80 µg/mL, while average percentage control growth of Adriamycin on HeLa is −21.2 on 10 µg/mL, −4.7 on 20 µg/mL, 5.0 on 40 µg/mL, −1.5 on 80 µg/mL.

As shown in ►Fig. 6, the average percentage control growth of V2S2 on human breast cancer cell line (MCF-7) is 24.1 on 10 µg/mL, 22.6 on 20 µg/mL, 19.0 on 40 µg/mL, 22.6 on 80 µg/mL, while average percentage control growth of Adriamycin on MCF-7 is 5.1 on 10 µg/mL, 2.5 on 20 µg/mL, −17.6 on 40 µg/mL, −20.6 on 80 µg/mL. The average percentage control growth of V2S2 on human hepatoma cell line (Hep-G2) is 14.8 on 10 µg/mL, 10.5 on 20 µg/mL, 9.9 on 40 µg/mL, 4.6 on 80 µg/mL, while average percentage control growth of Adriamycin on Hep-2 is −39.9 on 10 µg/mL, −46.5 on 20 µg/mL, −53.3 on 40 µg/mL, −44.1 on 80 µg/mL as shown in ►Fig. 7.

As shown in ►Fig. 8 average percentage control growth of V2S2 on human pancreatic cancer cell line (MIA-PA-CA-2) is −20.2 on 10 µg/mL, −32.7 on 20 µg/mL, −53.9 on 40 µg/mL, −55.4 on 80 µg/mL, while average percentage control growth

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**Fig. 7** Effect of different concentrations of V2S2 and Adriamycin on human hepatoma cell line Hep-G2.
of Adriamycin on MIA-PA-CA-2 is \(-39.4\) on 10µg/mL, \(-51.3\) on 20 µg/mL, \(-71.4\) on 40 µg/mL, \(-87.4\) on 80 µg/mL. However, the average percentage control growth of V2S2 on monkey kidney normal cell line (Vero) is \(-7.2\) on 10 µg/mL, \(-7.4\) on 20 µg/mL, \(-7.6\) on 40 µg/mL, \(-14.8\) on 80 µg/mL, while average percentage control growth of Adriamycin on Vero is \(-21.2\) on 10 µg/mL, \(-29.8\) on 20 µg/mL, \(-32.3\) on 40 µg/mL, \(-27.7\) on 80 µg/mL as shown in – Fig. 9.

**Discussion**

Cancer is a broad term and it describes the disease that results when cellular changes cause the division of cells and uncontrolled growth.\(^{11}\) Medicinal plants have shown to exhibit tremendous potential in prevention of several diseases owing to the anti-inflammatory, anticancer, antiviral, antimicrobial, immunomodulatory, apoptotic activity. Previous studies have shown notable anticancer activity of different plant extract against several cancer cell lines. Earlier findings elucidate potent anticancer activity of methanolic extract of *Eclipta alba* (L.),\(^{12}\) *C. cinereum*,\(^{13}\) and ethanolic extract of *Annona squamosa*.\(^{14}\) V2S2, the coded drug, has been prepared thoroughly considering the texts and studying anticancer activity of the herbs based on available literature. The control drug Adriamycin is chosen as it is proven anticancer drug that is commonly used as a chemotherapeutic agent. The experiment is performed according to guidelines of NCI and as per guideline, in in vitro experiment the value of GI50 \(\leq 20\) µg/mL is considered to demonstrate activity in case of extracts, while in case of pure compound, the value is \(\leq 10\) µg/mL. In this experiment, the GI50 of study drug (V2S2) has been found \(\leq 10\)µg/mL, which means that the V2S2 demonstrates high anticancer activity on almost all taken human cancer cell line in the experiment. Moreover, in vitro monkey kidney normal cell line study drug V2S2 shows same cytotoxicity as shown by control drug Adriamycin. Thus, V2S2 may be proved best anticancer drugs in future without any toxicity or with minimum toxicity then conventional drug. This study revealed the potential inhibitory activity of V2S2 on different cell lines. However, further experimentation will be required to explore the mechanistic underpinning of anticancer activity conferred by the extract.
Conclusion

From the study, we conclude that both study and control drug demonstrate anticancer activity in COLO-205, HOP-62, K-562, Ishikawa, HeLa, MCF-7, SCC-40, Hep-G2, and MIA-PA-CA-2. However, cytotoxicity activity of both study and control drug is more or less same. The more vigorous study on the herbal extract should be done for the development of herbal chemotherapy that has less cytotoxicity as compared with conventional chemotherapeutic agents. Thus, the V2S2 needs more evaluation and experiments for establishing it as a potent anticancer drug in future.

Financial Support and Sponsorship
None.

Conflict of Interest
None.

References