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# SYSTEMATIC REVIEW ON THE COMPARISON OF PRACTICALITY OF H9C2 AND HL-1 CELL LINE INVITRO MODELS IN TESTING THE ROS SCAVENGING ACTIVITY OF AYURVEDIC CARDIOPROTECTIVE MEDICINES

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**ABSTRACT**

A new initiative "Global Hearts" launched by WHO aims to beat back the global threat of cardiovascular diseases – the leading cause of death worldwide. By analysing different cardiovascular diseases (CVDs) the main pathophysiology behind is; oxidative stress generated by the increased production of reactive oxygen species (ROS). The ROS scavenging activity of medicines can be tested through invitro research. H9C2 cell line and HL-1 cell line are popularly used invitro models for testing the cardiotoxic and cardioprotective study of medicines. The administration of antioxidants helps to scavenge these ROS and can be utilized as both preventive and curative aspect of cardiovascular diseases. In Ayurveda, many single drugs and polyherbal preparations are mentioned in *hridroga chikilsa* (treatment for heart diseases) with a natural antioxidant supply. Among them, some were tested through invitro studies. This systematic review tries to compare the practicality of H9C2 cell line and HL-1 cell line invitro models in testing the ROS scavenging activity of Ayurvedic cardioprotective medicines and this review was completed by the comprehensive analysis of published research works in PubMed database and google scholar. The review of the literature was arranged systematically, guided by the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) flow diagram. The review was concluded by comparing the number of available research works on both cell line invitro models used in the study of Ayurvedic cardioprotective medicines. The H9C2 cell line model was found to be more utilized for testing the ROS scavenging activity of Ayurvedic cardioprotective medicines.

**KEYWORDS:** H9C2 cell line, HL-1 cell line, Invitro study, Reactive oxygen species, Ayurvedic medicine

## INTRODUCTION

Cardiovascular diseases (CVDs) are the major illness associated with the heart and blood vessels. According to WHO, CVDs are the leading causes of death worldwide. From CVDs, an estimated 31% of all deaths globally and causing approximately 17.9 million deaths each year.[1]

Oxidative stress generated during excessive accumulation of reactive oxygen species (ROS) is responsible for the pathophysiology of various cardiovascular diseases like myocardial infarction, atherosclerosis.[2] These diseases lead to life-threatening conditions such as heart failure. In the process of mitochondrial oxidative phosphorylation, ROS generated within the cells, or they may also develop from interactions with exogenous sources such as xenobiotic compounds. When ROS overwhelm the cellular antioxidant defence system, whether, through an increase in ROS levels or a decrease in the cellular antioxidant capacity, oxidative stress occurs.[3] Many epidemiological studies have observed that an increase in antioxidant level declines the clinical expression of Coronary artery disease.[4] The research studies for the intervention of proper antioxidants suitable for cardiovascular diseases is continuing. Preclinical researches like invitro and invivo studies are satisfactory for studying the ROS scavenging activity of cardioprotective medicines. Among them, invitro studies have less ethical issues. Invitro studies are included as an initial step in the evidence pyramid of research. Invitro pharmacology means research of the biological effects of drugs and pharmaceuticals, conducted outside of living organisms.

H9C2 cell line and Hl-1 cell line invitro models are popularly used for the invitro study of the cardioprotective effect of medicines against oxidative damage. The H9C2 cell line is a subclonal line of the original clonal cell line derived from embryonic BD1X rat heart tissue by Kimes and Brandt. The morphological parameters of their cells resemble immature embryonic cardiomyocytes therefore this cell line is commonly used in numerous invitro studies. H9C2 cell line is particularly used for studying the mechanisms of myocyte damage, and assessment of toxic effects of studied compounds on apoptosis and necrosis in cardiac myocytes. Embryonic H9C2 cardiomyocytes proliferate well in invitro

conditions, allowing relatively easy culturing.[5] HL-1 cardiomyocyte cell line is derived from mouse atria, utilized to study the oxidative damage.[6] HL-1 cells represent a cardiac myocyte cell line that can be repeatedly passaged and maintain a cardiac-specific phenotype.[7]

According to the World Health Organization, about 70 – 80 % of the world population depends on non-conventional medicines for their health care.[8] The goals of "WHO Traditional Medicine (TM) Strategy 2014–2023" are to promoting the safe and effective use of Traditional and complementary Medicine by regulating, researching and integrating medicinal products, practitioners and practice into health systems.[9] In Ayurveda many single drugs and polyherbal preparations are mentioned in *hridroga chikilsa* (treatment for heart diseases) with a natural antioxidant supply. The preclinical and clinical research studies on the cardioprotective effect of Ayurvedic medicines are continuing.

A study conducted on H9C2 cells and HL-1 cells about energy metabolism patterns shows that H9C2 cells are more similar to primary cardiomyocytes than HL-1 cells and H9C2 cells can be a more suitable model to study cardiac ischemia-reperfusion injury.[10] H9C2 cell line and HL-1 cell line were also utilized for testing the Ayurvedic cardioprotective medicines. There is a lack of such comparison studies on the practical feasibility of these cell lines in testing the Ayurvedic cardioprotective medicines. The main objective of this review is to compare the practicality of H9C2 cell line and HL-1 cell line invitro models in testing the ROS scavenging activity of Ayurvedic cardioprotective medicines.

## METHODS

### Inclusion criteria

Among the invitro studies on cardioprotective effect of Ayurvedic medicines using H9C2 cell line and HL-1 cell line invitro models, ROS related studies were only included. Studies on single drugs, formulations, active components extracted from Ayurvedic drugs were included for the review.

## Exclusion criteria

Duplicated, data unrelated to the objective, articles without full text available were excluded from the systematic review.

## Identification of studies

A systematic review of the PubMed, google scholar, and Cochrane databases was performed. Search on these electronic databases from inception to 23rd December 2020. The keyword 'H9C2 cell line and Ayurveda' and 'HL-1 cell line and Ayurveda' were used for searching in databases like PubMed, Cochrane and google scholar. In PubMed, the articles were sorted by best match and full text. In google scholar, the search results were sorted by relevance and include both patents and citations.

## Study selection

Studies that matched the inclusion criteria were retrieved for the comprehensive analysis. Duplicates removed after screening based on titles and abstracts.

## Quality assessment

The review of the literature was completed systematically, guided by the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) flow diagram.[11] This systematic review aims to compare the practicality of H9C2 cell line and HL-1 cell line invitro models in testing the ROS scavenging activity of Ayurvedic cardioprotective medicines. The comparison in terms of practicality was done based on the number of studies gathered from PubMed, Google Scholar and Cochrane in each cell line invitro model.

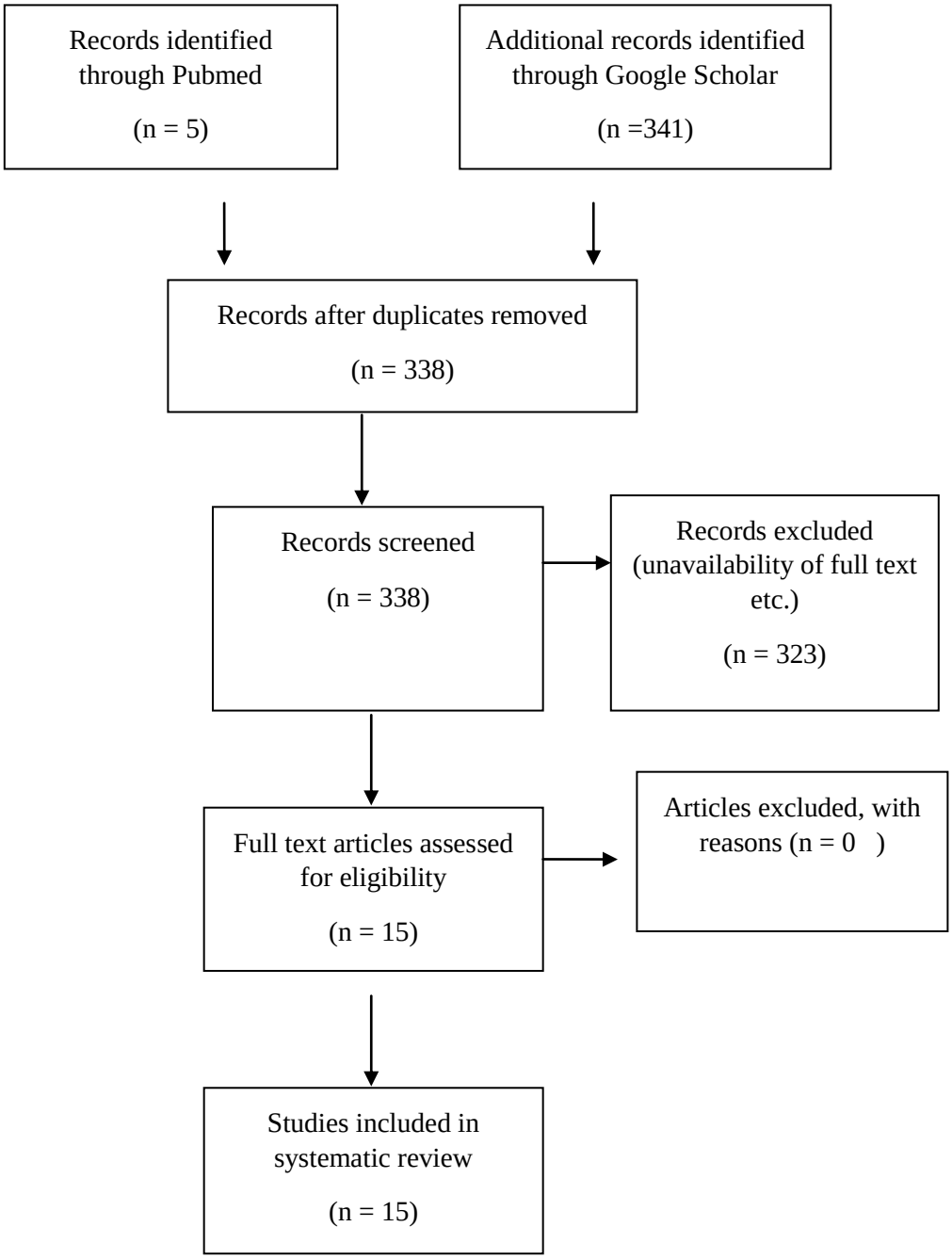


**Fig.1. Diagrammatic representation of systematic review process**

## RESULT

A total of 346 titles and abstracts were reviewed (5 from the PubMed database; 341 from google scholar; the number of search results from the Cochrane database is zero). After the removal of duplicates (*n* = 8), 338 articles have remained. The titles and abstracts of 338 records were screened. Of these, 323 articles were identified as irrelevant and excluded because these articles did not meet the inclusion criteria and due to the unavailability of the full text. Finally, 15 articles were screened for comprehensive analysis. After cross-checking, the authors agreed upon 15 articles to be included because they meet the inclusion criteria.

A study was conducted in *Bacopa monnieri*, a medicinal Ayurvedic herb on HL-1 cell line invitro model. It was evidenced that *B.monnieri* reduced $I_{Ca,\ L}$ in HL-1 cardiac cells.$Ca^{2+}$ is an important mediator of ischemic cell damage and in cardiac muscle, $Ca^{2+}$ influx pathway is mainly through L-type $Ca^{2+}$ channels.[12] Both ICa,L and ROS mediated signalling pathways cross-talk with each other in cardiac myocytes. For example, there is considerable evidence that the function of the L-type Ca2+ channel is influenced by the redox state of the cell, leading to intracellular calcium overload. Conversely, calcium overload damages the mitochondrial function and enhance the intracellular ROS production.[13]

*Boerhaavia diffusa* is one of the cardiotonic plant known as '*punarnava*' in Ayurveda studied against angiotensin II-induced hypertrophy in H9c2 cardiac myoblast cells.[14]

*Nardostachys jatamansi* is a well-known medicinal herb widely used in Ayurvedic medicines for the treatment of cardiovascular disorders. In this study, the total volatile oil from root and rhizome was isolated. The cytoprotective effect of this volatile oil against tBHP (tert-Butyl hydroperoxide)-induced H9c2 cardiomyocyte was studied.[15]

Dalspinin isolated from *Spermacoce hispida (vasuka)* exhibits protection against hypoxic injury in an invitro hypoxic model of cultured H9c2 cardiomyocytes. Cobalt chloride ($CoCl_2$) was used to induce hypoxia in H9c2 cardiomyoblasts.[16]

Doxorubicin was used to induce oxidative stress in H9C2 cell line in the following studies;

- *Glycyrrhiza glabra* (Licorice) root extract attenuates doxorubicin-induced cardiotoxicity via alleviating oxidative stress and stabilising the cardiac health in H9c2 cardiomyocytes.[17]
- Protective effect of guggulsterone against cardiomyocyte injury induced by doxorubicin invitro.[18]
- Pummelo Protects Doxorubicin-Induced Cardiac Cell Death by Reducing Oxidative Stress, Modifying Glutathione Transferase Expression, and Preventing Cellular Senescence.[19]
- Therapeutic Potential of Fermented Papaya Preparation in Doxorubicin Induced Toxicity.[20]
- Cardioprotective Potentials of Plant-Derived Small Molecules such as arjunolic acid, gingerol, curcumin, eugenol etc against Doxorubicin Associated Cardiotoxicity.[21]

$H_2O_2$ was used to induce oxidative stress in H9C2 cell line in the following studies;

- An invitro study on strengthening antioxidant defence & cardio protection by *Piper betle.*[22]
- A Study was conducted on *Anethum graveolens* L. seed extract in alleviating cardiovascular complications using invitro and invivo Experimental Models.[23]
- *Thraatchathi Choornam*, protects cardiomyocytes against oxidative stress.[24] *Thraatchathi choornam*, a Siddha polyherbal formulation has the traditional claim for the management and treatment of cardiovascular diseases. *Thraatchathi Chooranam* is one of the herbal rejuvenating medicines which contains equal portions of 32 herbs such as *Vitis vinifera, Phoenix dactylifera, Cyperus rotundus, Piper wallichi, Santalum album, Oryza sativa, Curcuma anguistifolia, Elattaria cardamomum, Cuminum cyminum, Vetiveria zizonoides, Zingiber officinale [dried], Piper nigrum, Piper longum, Terminalia chebula, Terminalia bellarica, Embilica officinalis, Pavonia odorata, Costus speciosus, Glyzhirrizha glabra, Pavonia zeylanica*, *Tinospora cordifolia, Gmeliana asiatica, Tribulus terrestris, Plectranthus vittiviroides, Coccinium fenestratum, Nymphaea pubaecens, Syzigium aromaticum, Curcuma aromatica, Crocus sativus, Kaempferia galangal, Neliumbo nucifera and Sitramalli*.[25]

Effects of pomegranate seed oil was studied on oxidant/antioxidant balance in heart and kidney homogenates and mitochondria of diabetic rats and high glucose-treated H9c2 cell line.[26] Suppression of isoproterenol induced hypertrophy in H9C2 by traditional indian medicine *Yogendra Ras*.[27]

Pretreatment of *Tribulus terrestris* L. causes anti-ischemic cardioprotection in ischemia-induced H9C2cell line. To induce ischemia, H9c2 cells were maintained at 37 °C, 0.1% $O_2$, 5% $CO_2$, 95% $N_2$ in a hypoxia incubator, in an ischemic buffer for 1 h.[28]

| SL.NO | MEDICINE WITH CARDIOPROTECTVE EFFECT | TEST MATERIAL IN CULTURE MEDIA | DRUG USED TO INDUCE OXIDATIVE STRESS IN H9C2 CELL LINE |
|---|---|---|---|
| 1 | *Boerhaavia diffusa* | Ethanol extract of the whole plant | Angiotensin II |
| 2 | *Nardostachys jatamansi* | Volatile oil from root and rhizome | tBHP (tert-Butyl hydroperoxide) |
| 3 | *Spermacoce hispida* | Dalspinin isolated from *S.hispida* methanolic extract | Cobalt chloride ($CoCl_2$) |
| 4 | *Glycyrrhiza glabra* | root extract | Doxorubicin |
| 5 | Guggulsterone | Steroid extracted from *Commiphora myrrha* | Doxorubicin |
| 6 | Pummelo | Fruit extract | Doxorubicin |
| 7 | Fermented Papaya Preparation | - | Doxorubicin |
| 8 | *Piper betle* | Dried leaf ethyl acetate extract | H202 |
| 9 | *Anethum graveolens* | Seed extract | H202 |
| 10 | *Thraatchathi Choornam* | Powder | H202 |
| 11 | Pomegranate | Seed oil | Glucose |
| 12 | *Yogendra Ras* | Powder | Isoproterenol |
| 13 | *Tribulus terrestris* | The methanol extract of the whole plant without fruits | 95% $N_2$, ischaemia buffer |

**Table.1. Summary of the results**

## DISCUSSION

This review is a small attempt to provide an idea about the invitro cell line models (H9C2 and HL-1) used in the cardioprotective study, the role of ROS in the causation of cardiovascular diseases, ROS related invitro studies conducted in Ayurvedic cardioprotective medicines using these cell line models.

As per the review, it is evident that both the H9C2 cell line and HL-1 cell line are utilized to test the Ayurvedic cardioprotective medicines. Based on the inclusion criteria the ROS related studies are only selected for review. By analysing the studies H9C2 cell line was utilized for testing the ROS scavenging activity of Ayurvedic cardioprotective medicines. But the HL-1 cell line was used to study the preventive role of medicine in the generation of ROS. Among the 15 included studies, 14 utilized the H9C2 cell line for testing the Ayurvedic cardioprotective medicines. But only one study on the HL-1 cell line was gathered through the online search. From the collected data it is evident that the H9C2 cell line is utilized mostly for ROS related studies on Ayurvedic cardioprotective medicines than the HL-1 cell line invitro model. In this review, the practicality or feasibility of both cell line models was only compared based on the number of studies conducted. As per the review, it is understood that the H9C2 cell line invitro model is practically feasible in testing the ROS scavenging activity of Ayurvedic cardioprotective medicines.

There is a need to apply new strategies of research methodology in the field of Ayurveda, for making it more evidence-based. Ayurveda follows the deductive type of research; here studies are needed only to confirm the postulated theories. This confirmation can be achieved through the observations. Evidence in research means the confirmation or verification of facts through well-organized experiments. The H9C2 cell line and HL-1 cell line are the tools for invitro cardioprotective studies and Ayurvedic drugs with cardioprotective effect are the study sample to be proved. As per the systematic review of the available search results, it is evidenced that, H9C2 cell line is popularly used as an invitro study model for testing the cardioprotective effect of Ayurvedic medicines.

Limitations:

- Full texts are not available in most of the studies
- Only one study is available on the HL-1 cell line so the strength of the quality of comparison may be less.

## CONCLUSION

Overproduction of reactive oxygen species and oxidative stress are responsible for the pathophysiology of various cardiovascular diseases. Preclinical researches like invitro and invivo studies are enough for studying the ROS scavenging activity of cardioprotective medicines. Among them, invitro studies have less ethical issues.

H9C2 cell line and Hl-1 cell line invitro models are popularly used for the invitro study of the cardioprotective effect of medicines against oxidative damage. As per the existing knowledge, H9C2 cells are more similar to primary cardiomyocytes than HL-1 cells and H9C2 cells can be a more suitable model to study cardiac ischemia-reperfusion injury. H9C2 cell line and HL-1 cell line were utilized for testing the Ayurvedic cardioprotective medicines also. There is a lack of such comparison studies in Ayurvedic cardioprotective medicines. The main purpose of this review is to compare the practicality of these cell line invitro models in testing the ROS scavenging activity of Ayurvedic cardioprotective medicines.

A review of the currently available literature in PubMed and Google Scholar shows that the H9C2 cell line is feasible as an invitro study model compared to the HL-1 cell line for determining the ROS scavenging activity of Ayurvedic cardioprotective medicines. Therefore this cell line can be further utilized for testing the cardioprotective effect of Ayurvedic medicines.

A better understanding of the molecular basis of an Ayurvedic formulation is the proven evidence of our valuable treatment system. Many questions are arising about the rationality and relevance of the application of invitro researches in Ayurvedic drugs. The above-said data suggests that invitro studies have a proven role to analyse the cellular level activities of Ayurvedic medicines also. Therefore these studies contribute much more evidence to the scientific society.

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