Pharmaceutico-Analytical Study of *Panchanana Rasa* and Evaluation of Its Anti-inflammatory Activity in Myocarditis using LPS Induced Inflammation in Cardiac Cell Line H9C2.

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Abstract

Introduction: Now a day, Sudden deaths due to Cardiac Failure has been seen in young adults and children. Myocarditis occurs due to acquired cardiomyopathy where myocardium (heart muscles) gets inflamed because of any viral infections or systemic inflammatory conditions caused by autoimmune disorders. It is also difficult to diagnose Myocarditis as its symptoms are unusual. In *Ayurveda, Panchanana Rasa* has been mentioned to treat *Hridroga* and *Dravya* used in this *Kalpa* has found to be cardioprotective and anti-inflammatory activity. As there is need to develop standard procedure of formulation which can be cost effective along with efficacy, we are doing in vitro study on H9C2 Cardiac cell line.

Aim and objectives: To perform pharmaceutical and analytical process of *Panchanana Rasa* & evaluation of its Anti-inflammatory activity in Myocarditis using LPS Induced Inflammation in Cardiac Cell line H9C2.

Materials and methods: *Panchanana Rasa* was prepared as per classical reference mentioned in *Hridroga Adhikar of "Bhaishajyaratnavali*." *Panchanana Rasa* has been assessed with analytical tests such as XRD, SEM and other physio-chemical parameters. The experimental study has been done to evaluate anti-inflammatory activity of *Panchanana Rasa* on H9C2 (2-1) Cardiac cell.

Result: On evaluating cytotoxic effect of *Panchanana Rasa* through XTT assay it was found that *Panchanana Rasa* do not cause toxic effect on H9C2 cell. In DPPH Assay it is found to be more potent anti-oxidant than ascorbic acid.

Conclusion: The LC 50 value of *Panchanana Rasa* found to be 1106.1µg/ml, which is extremely high and thus it can be concluded that the drug is safe to use for further therapeutic purposes. After assessment of XTT assay and DPPH assay for free radicle scavenging, *Panchanana Rasa* can possibly act as anti-inflammatory drug for further immune-modulatory activity. A small step to fill the research gap in the treatment aspect of Myocarditis from Ayurvedic point of view has been done.

Keywords: Myocarditis, Anti-inflammatory activity, *Rasaushadhi*, *Panchanana Rasa*, H9C2 cell line, *Khalviya Rasayana.*

INTRODUCTION:

Ayurveda, the art of living and the science of life is serving the man kind since antiquity. Its origin dates back to *Veda* where it is probably available in the form of religious preaching. A major portion of *Ayurveda* is devoted to teach the human personnel, his way of life, behavior in particular, both personal and social.[1]Globalized and Modernized practices derived from *Ayurveda* traditions are a type of complementary or alternative medicine.[2,3]

Rasa shastra is a pharmaceutical branch of Indian system of medicine which mainly deals with the minerals, products of animal origin, metals, and their use in therapeutics.[4]The word *Ras shastra* refers to term which is related to "Science of Mercury" in Chemistry. *Rasa shastra* is a specified branch of *Ayurveda,* different aspects of classification, *Rasa Dravya's* processing of metal and minerals products with a note on the methods used during different period.[5]

Bhaishajya Kalpana, which deals with drug awareness, including identification, acquisition, processing, preparation, and application, is one of the most significant sections of *Ayurveda*.[6]The incidence of diseases with an inflammatory aetiology has gone up recently. In addition to treating the illness, medications used for therapeutic management of certain inflammatory illnesses also prevent major, perhaps fatal side effects. In addition, they are expensive and not always readily available. In this regard, advancements in the study and application of medicinal herbs have ushered a new chapter in the preventative and treatment of inflammatory illnesses.[7]

Myocarditis is a heterogeneous type of disease with various kind of symptoms starting from mild chest discomfort to cardiogenic shock. As it often resembles other common disorders, such as coronary artery disease (CAD), diagnosis may be challenging.[8] The majority of myocarditis cases are brought on by autoimmune diseases, medications, toxic chemicals, or infectious pathogens.[9] Over the years, many diagnostic tests have developed to identify patients suffering from myocarditis. Nowadays, a diagnosis of myocarditis contains clinical, laboratory, imaging and histological parameters.[10]Endomyocardial biopsy (EMB) is the gold standard for diagnosis of Myocarditis[11,12]Cardiac magnetic resonance imaging (CMR) is considered a non-invasive alternative in patients with suspected Myocarditis.[13,14] Post Covid vaccination induced Myocarditis has been seen in young males. Young males between 16 and 29 years of age had the highest incidence of post-mRNA vaccine myocarditis at 10.7 cases per 100,000[15,16]

The *Kharaliya Rasayana* stands prior in comparison with *Parpati, Pottali* & *Kupipakwa Rasayana* in terms of its popularity, wide range of therapeutic utility & ease of preparation. *Parpati, Kupipakwa* & *Pottali Rasayana* are limited in numbers but the *Kharaliya Rasayana* are innumerable and most suitably indicated in vast majority of disease condition. Since these formulations are completely prepared and obtained as end products in *Khalvayantra* by the act of trituration, they are called as *Kharaliya* or *Khalviya Rasayana*.[17] Shelf life of *Kharaliya Rasayan* is infinite for *Rasayoga* [18] and for *Rasa, Uprasa, Bhasma, Guggul* with herbal drugs is 5 yrs. [19]The principle used in preparation of *Kharaliya Rasayana* is based on the principle of *Mardana* and *Bhavana* (Trituration). *Mardana* is the

process of trituration of drugs to a find state of division with or without prescribed liquid. *Bhavana* is the process by which powders of drugs are ground to a soft mass with liquid substances and allowed to dry.[20] The *Rasausadhis* are known for smaller dosage & quick results. *Rasausadhis* don't cause any nauseating sensation during consumption. These medicines provide quick results and are useful in majority of disease conditions.[21]

Panchanana Rasa mentioned in *Bhaishajyaratnavali* is indicated in treating *Hridroga*.[22]*Bhavna Dravya* used in *Panchanana Rasa* are found to be Cardioprotective , Anti-inflammatory, Immuno-modulatory and Antioxidant in various preclinical studies.[23-27]Pharmaco therapeutic advantage of *Kajjali Kalpa* as per reverse pharmacological observation is, when compared to other pharmacological forms, the Herbo mineral compound classically prepared by using Kajjali possesses large stay in intestinal mucosa.[28]The long alarming of the intestinal *Kajjali* compound better crosses BIB (Blood intestinal barriers) and hence more pharmacological activity is possible by using *Kajjali Rasayanas*.[29]

Insufficient literature regarding conceptual, pharmaceutical, analytical studies of *Panchanana Rasa* has formed an empirical knowledge gap hence we have conducted In vitro study in order to develop future clinical study regarding this *Kalpa* for its better therapeutic use.

MATERIALS & METHODS:

Collection and Authentication of raw drugs:

All raw drugs were procured from authenticated vendor and further authenticated from experts of *Rasashastra* dept. of our institute and botany dept. RTMNU Nagpur.

Raw materials used for preparation of *Panchanana Rasa* were *Parada*[30] (Mercury) and *Gandhak* [31](Sulphur). Media used for *Bhavna* were *Yashtimadhu Kwatha, Amalaki Swarasa*[32] ,*Draksha* and *Kharjura Kwatha*[33]. *Panchanana Rasa* was prepared as per reference mentioned in *Bhaishajyaratnavali* in *Hridrogadhikar Prakaran.*

Pharmaceutical study:

1. ***Parada Shodhana*[34] :** *Parada Shodhana* has been done with help of *Rasona*[35] *Kalka.* As *Paradsamhita*, required quantity of *Ashuddha Parada* (Hydrargyrum) triturated with equal amount of *Rasona Kalka* (Allium Sativum) in *Khalvayantra*. *Parada* gets divided into small globules. Trituration done continue for 6 hrs. for 7 days , then *Shuddha Parada* obtained by *Parada Prakshalan* with *Koshna Jala*.[36]

2. ***Gandhaka Shodhan*[37]:** *Gandhak Shodhan* done by *Dhalan* method in *Godugdha* [38] for 7 times as per the reference *Ras Ratna Samuchhay*. The required quantity of *Gandhak* melted into the *Ghee* [39] taken in the steel *Darviyantra*. This molten *Gandhak* then poured into the container with milk. Once the *Gandhak* got solidified, it removed from the milk, washed with hot water and further processed for *Shodhan* for six times. During this process, the fat soluble impurities are dissolved in the *Ghee*; water soluble impurities are dissolved in the milk while physical impurities are retained by the filter cloth.[40]

3. ***Kajjali* preparation[41] :** 200 g dried *Gandhak* powder was taken in *khalvayantra* and trituration was done to make fine powder. *Shuddha Parad* was weighed 200 g accurately poured in *Khalvayantra*. Trituration was done continuously till the mixture of *Parada* and *Gandhak* became homogenous. Trituration was continued till the mixture became fine and black in colour and passed all the classical parameters like *Kajjalabh, Nishchandratva [42], Rekhapurnatva [43]*and *Varitar.*

4. **Preparation of *Panchanana Rasa* :** Prepared *Kajjali* is levigated with *Yashtimadhu Kwatha, Amalaki Swaras, Draksha Kwatha* and *Kharjura Kwatha* each for one day. During levigation, the minute drug particles come in contact with the liquid media and during grinding heat are produced. This may result into occurrence of chemical reaction between the *Kajjali* and *Bhavna Dravya.*[44] *Bhavana* continued till the dough became suitable for *Vati Nirman* and then *Vati* was prepared. Then it was shade dried. Later *Panchanana Rasa* was stored in *Dhupit* glass container.

AShuddha Parada

Mardan in Rason Kalka

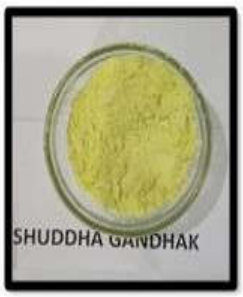
Parada disintegrates into fine particles

Shuddha Parada

Ashudhha Gandhaka melted in ghee

Melted Gandhak poured in Godugdha

Gandhak in koshna jal



Shuddha Gandhak

Table No. 1 showing Dravya used in *Panchanana Rasa* formulation:

Ingredients	Latin name	Part used
Shuddha Parada	Hydrargyrum (Hg)	-
Shuddha Gandhaka	Sulphur (S)	-
Amalaki Kwatha[45]	Phyllanthus emblica	Fruits
Draksha Kwath[46]	Vitis vinifera	Fruits
Yashtimadhu Kwath[47]	Glycyrrhiza glabra	Stem
Kharjura Kwath[48]	Phoenix sylvestris	Fruits

Analytical Study :

In this study , *Panchanana Rasa* was evaluated through following analytical parameters

A) Organoleptic characters[49] - like colour, odour, taste, texture and weight.

B) Physiochemical parameters like-

1. pH[50]
2. LOD[51]
3. Acid insoluble ash[52]
4. Water soluble ash [53]
5. Total ash [54]
6. XRD[55]
7. SEM[56]

Experimental Study:

1. Cell culture :[57]

Cardio myoblasts derived from the embryonic rat heart, H9C2 (2-1) cell line was purchased from National Centre for Cell Science (NCCS), Pune, India. Cells were maintained in Dulbecco's Modified Eagle Media and 10% Foetal bovine serum (FCS) and 1% antibiotic anti-mycotic solution (Himedia, India), containing 10,000 units penicillin, 10mg streptomycin and 25lg amphotericin B/ml of culture medium as recommended supplier of cell line. The cells were routinely incubated in poly-L-lysine coated tissue culture flasks in humidified atmosphere of 5% CO2 and 95% air at 37^0C**.**

2. Dissolution of *Panchanana Rasa* for Cell line:

The *Panchanana Rasa* was in powder form. 100 mg of powder was dissolved in 10ml of respective Media i.e. DMEM.[58] The mixture of drug and media (DMEM) was allowed to get dissolved for 24 hours and then next day it was filtered through a 0.25 micron syringe filter. The filtrate which was collected was used as a stock of 10ug/ml for further experimental purpose.

3. Lipopolysaccharide (LPS):[59,60]

In this project, LPS (Lipopolysaccharide) was used to stimulate the inflammatory in cardiomyocytes and then *Panchanana Rasa* was added to evaluate its anti-inflammatory activity. Lipopolysaccharide is the main component of the outer membrane in Gram-negative bacteria which acts as a crucial pathogenic factor in contributing to numerous dysfunctions LPS stimulates cells through Toll-like receptor 4 (TLR4), causing the release of inflammatory cytokines and up regulation of co-stimulatory molecules on antigen presenting cells. As LPS is added in the PBMC cells,[61] it induces inflammation and thus increases the cell count of antibodies as a response to the inflammation caused.

4. Measurement of cell viability: [62]

Trypan blue exclusion assay

Trypan blue staining is a simple way to evaluate cell membrane integrity and thus cell viability. The isolated and washed lymphocytes were checked for viability and cell count using a haemocytometer. The purpose was to determine the cell density of viable cells in the culture. The viable and non-viable cell density can be determined with use of dye (Trypan blue) exclusion assay. The viable or living cells do not dye while non-viable or dead cells do because of disrupted cell membrane. Non-viable cells do not have the metabolic capability to expel the intruding dye. In brief 50 µl of washed cell suspension in a micro centrifuge tube was taken. 400µl PBS and 50 µl of trypan blue solution was added to the cell suspension and mixed thoroughly. Trypan blue-cell suspension was then transferred to a chamber of haemocytometer with cover slip using a micropipette. It was done carefully by touching the edge of the cover slip with the pipette tip and allowing the chamber to fill by capillary action.

Care was taken to neither overfill nor under fill the chamber. Viable cells were counted (colourless opaque) in all the four WBC counting chamber of haemocytometer under light microscope. Occasionally non-viable cells with blue stained nucleus were also observed.

Cell concentration per ml was calculated as follows:

Average number of cells in one large square x dilution factor* x 10 4

*Dilution factor is usually 2 (1:1 dilution with trypan blue), but may need to further dilute (or concentrate) cell suspensions.

10^4 = conversion factor to convert 10- 4ml to 1ml.

5. Measurement of cytotoxicity: [63]

The XTT assay was performed by seeding H9C2 (2-1) cells separately in a 48 well plate at a density of 15µl cells/well and *Panchanana Rasa* was added in four different concentrations like (100ug/ml, 50ug/ml, 250ug/ml, and 12.5ug/ml) and incubated for 48 hours to check the absence or presence of cytotoxic effect after addition of that. After 48 hours, 10µl of XTT was added to each well and the plate was incubated at 37°C for 4hrs. Inflammation induced using LPS was also evaluated after 48 hours of incubation using XTT assay. XTT measures cell viability based on the activity of mitochondrial enzymes in a live cell that reduces XTT to a highly water-soluble product, which is directly proportional to the number of living cells in the sample. The absorbance was measured at 450 nm. Toxicity was calculated comparing the viability of the control cells 100%. The LC50 value was calculated based on a nonlinear regression (curve fit).

6. DPPH Assay [64]

2,2-Diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich) was prepared at a concentration of 0.04 mg/ml in freshly prepared methanol. A standard solution of ascorbic acid (1 mg/ml) was also prepared in methanol. *Panchanana Rasa* was used at concentrations of 100 µg/ml, 5 µg/ml, 25 µg/ml, and 12.5 µg/ml in methanol to evaluate its antioxidant properties.

The DPPH radical exhibits strong absorbance at 517 nm (deep violet colour) due to its unpaired electron. When the radical pairs off in the presence of a free radical scavenger, the absorption diminishes, and the resulting discoloration is proportional to the number of electrons accepted. An equal volume of DPPH solution was added to different concentrations of drug dilutions, mixed, and incubated at room temperature for 30 minutes in the dark. The absorbance was then measured at 570 nm, with DPPH solution containing an equal volume of methanol as the reagent blank.

7. Photomicrographs

Photomicrographs was taken with the help of Inverted Tissue Culture microscope (Make-Metzer) to find out the morphological changes after addition of *Panchanana Rasa* in PBMCs and H9C2 (2-1) cells.

OBSERVATIONS AND RESULT:

Pharmaceutical study:

Table No 2 showing observations regarding *Parad* and *Shodhan* process:

Drug	Initial Wt.	Final Wt.	Weight loss	% of Wt. loss	Observations
Parada	500 g	480 g	20 g	4	*Shuddha Parada* obtained was silvery in colour , shiny and lustrous.
Gandhaka	500 g	478 g	22 g	4.4	*Shuddha Gandhaka* obtained was bright yellow in colour and granular in structure.

Table No 3 showing observations during *Kajjali* Preparations:

Day	Observations
1	As soon as we started trituration ,*Parad* was freely moving in *Khalwayantra*, but after 1 hour it gradually mixed with *Gandhak* and then turns into greenish black in colour. At the end of 3 hrs. most of the particles of *Parad* were seen mixed in *Gandhaka.*
3	*Parad* and *Gandhaka* were completely mixed and mixture was blackish grey in colour.
6	Droplets of *Parada* gets disappeared completely at this stage.
9	*Kajjali* in the *Kalawana* was dark black which passed *Rekhapurnatwa Pariksha*
12	*Kajjali* became *Shlakshna , Sukshma* in texture but some *Chandrika's* were seen there.
15	Black coloured *Kajjalabha* like paste with *Mrudu* and *Shlakshnatwa* property was obtained .The mixture (*Kajjali*) possesses *Nishchandra, Sukshmtwa* and *Rekhapurnta* properties

Table No 4 showing observation after *Kajjali* preparation:

Drug	Initial Wt.	Final Wt.	Wt. loss	% in wt. loss
Kajjali	**400 g**	**355 g**	**45 g**	**11.25**

Table No 5 Observation of *Bhavna Dravya* preparation for *Panchanana Rasa* :

Bhavna Dravya	Wt. of *Dravya* in (g)	Water used in (ml)	*Kwatha* obtained in (ml)	Duration required in (min)
Yashtimadhu	125	2000	250	45
Amalaki	500	100	300	30
Draksha	125	2000	250	45
Kharjura	125	2000	250	35

Table No 6 Observations obtained during preparation *Panchanana Rasa*

Sr No	Characters	Observations
1.	Initial wt. of *Kajjali*	200 g
2.	Final wt. of *Kajjali* after 4 *Bhavna Dravya*	220 g
3.	% wt. gain	10
4.	Colour	Blackish grey
5.	Odour	*Amla Gandhi*
6.	Taste	Not specific
7.	Texture	Soft and sticky

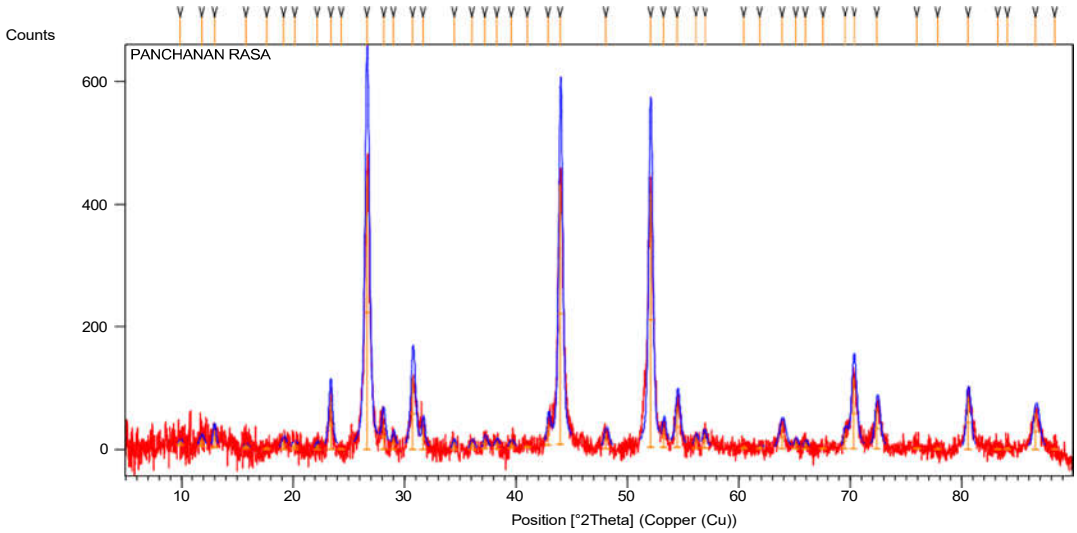
Analytical Study:

Physiochemical analysis:

Table No 7 showing results of modern parameters:

Sr No	Parameters	Result
1.	pH	4
2.	Water soluble ash	0.70 %w/w
3.	Acid insoluble ash	1.70 %w/w
4.	LOD	3.18 %w/w
5.	Total ash	3.23 %w/w

XRD Study: Graph No 1 : XRD Pattern of *Panchanana Ras*



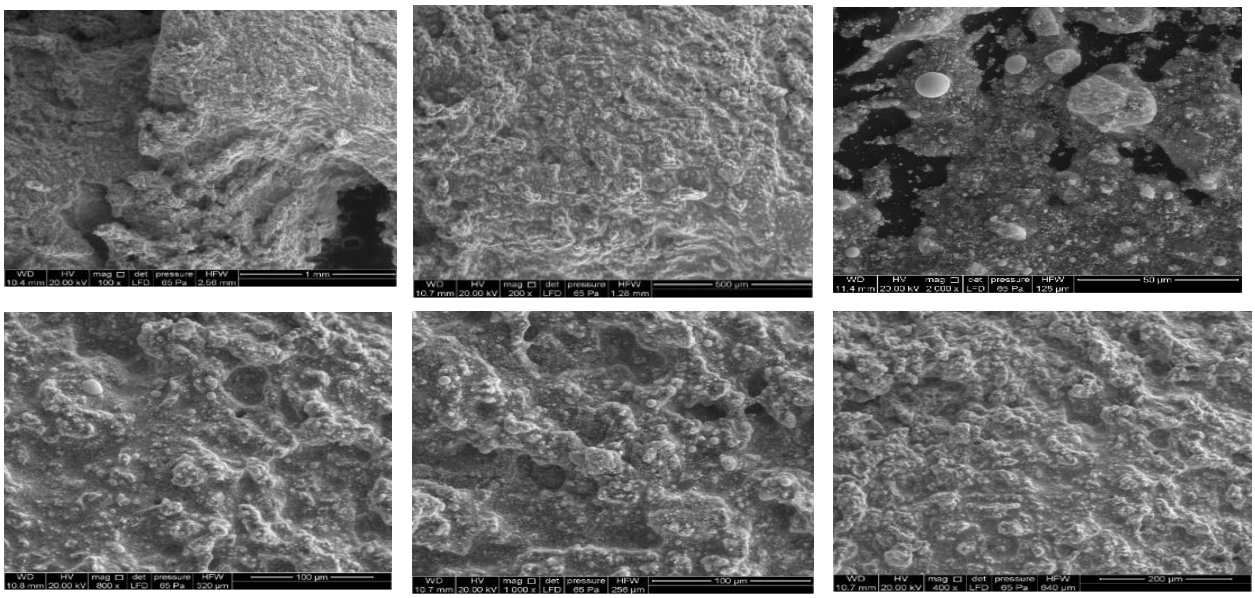
XRD study shows that *Panchanana Rasa* constitutes Cinnabar (Hg1S1) and Sulphur (S8) as a major compound.

SEM Study:

Table No 8 showing results of SEM :

Magnification	Particle size
100 x	1 mm
200 x	500 µm
400 x	200 µm
800 x	100 µm
1000 x	100 µm
2000 x	50 µm

Pictures showing results of SEM

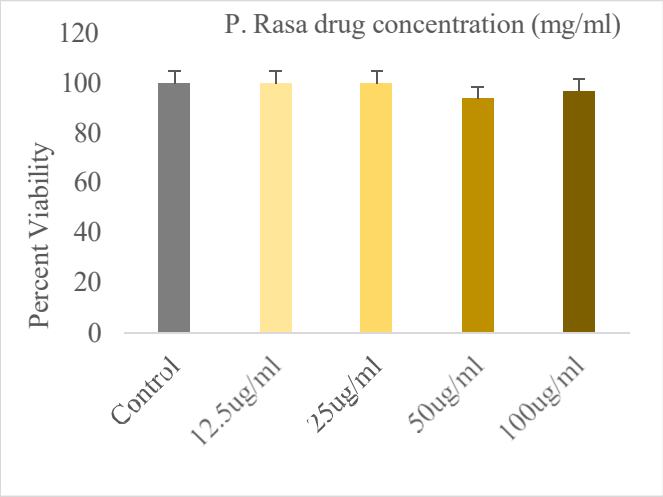


SEM images of *Panchanana Rasa* were captured at different magnification ranging from 100X up to 2000X. Smallest particle size was found to be ranging between 1mm at 100X magnification with Wd 10.4 mm and 50µm at 2000X magnification with Wd 11.4 mm.

Experimental study:

Result of cytotoxicity assay

Figure 1. represents the results of the cytotoxicity assay conducted to evaluate the cytotoxic effects *Panchanana Rasa* in H9C2 (2-1) cell line at various concentrations like 12.5µg/ml, 250µg/ml, 50µg/ml, and 100µg/ml. The primary objective of this assay was to determine whether these concentrations would induce any cytotoxic effects, thereby affecting cell viability. The findings revealed that *Panchanana Rasa* did not cause any toxic effects on H9C2 (2-1) and PBMCs cell line. Specifically, cell viability remained stable across all tested concentrations, with no significant decrease in viability observed ($P > 0.05$ vs. Control). This indicates that drug is non-toxic at the tested concentrations. The LC50 value of H9C2 (2-1) was found to be 1106.1µg/ml, which is extremely high and thus it can be concluded that the drug is safe to use for further therapeutic purposes.



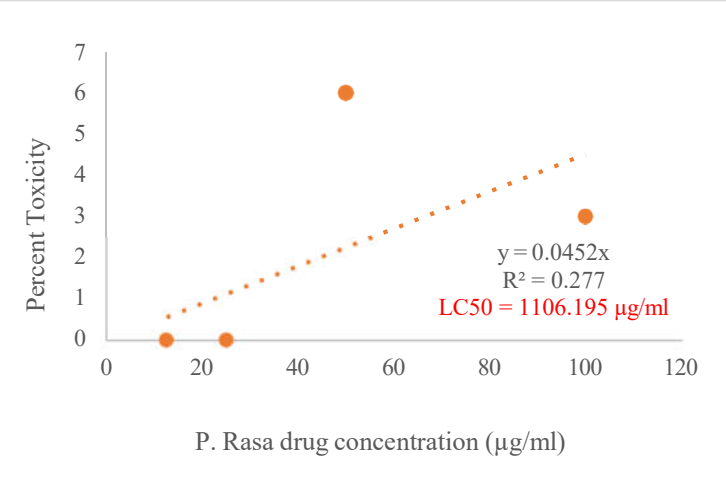


Figure 1 (i): Represents graph showing percent viability of H9C2 (2-1) cell line after treatment *of Panchanana Rasa drug* (dissolved in DMEM) at various concentrations like 12.5 µg/ml, 25 µg/ml, 50 µg/ml, and 100 µg/ml. **(ii):** Represents graph of LC50 calculation

Result of DPPH Assay:

The comparison of antioxidant activity between ascorbic acid (10 mg/ml) and *Panchanana Rasa* at various concentrations (12.5 µg/ml, 25 µg/ml, 50 µg/ml, and 100 µg/ml) reveals that *Panchanana Rasa* exhibits higher DPPH radical scavenging activity across all concentrations tested. Ascorbic acid, used as a standard, shows a 37% reduction in DPPH radicals, while *Panchanana Rasa* at the lowest concentration of 12.5 µg/ml demonstrates a 63% reduction. The scavenging activity increases with the concentration of *Panchanana Rasa*, reaching a peak

of 72% at 25 µg/ml. At higher concentrations, the scavenging activity remains significant but slightly decreases, showing 70% at 50 µg/ml and 67% at 100 µg/ml. This data suggests that *Panchanana Rasa* has potent antioxidant properties, with its maximum effectiveness observed between 25 µg/ml and 50 µg/ml. Interestingly, the scavenging activity does not increase proportionally with higher concentrations, indicating a possible saturation effect or optimal concentration range. This suggest that *Panchanana Rasa* is a more effective free radical scavenger than ascorbic acid at the concentrations evaluated in this study.

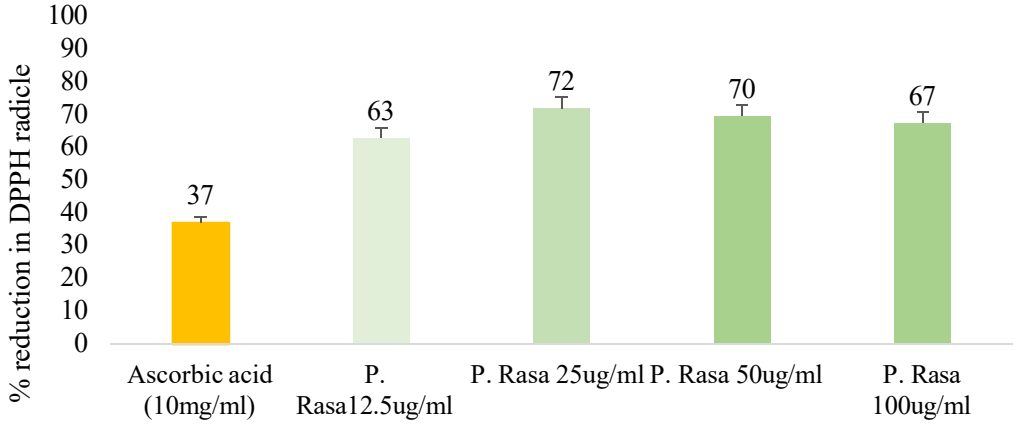


Figure 2: Represents graph DPPH radical scavenging activities of various concentrations of *Panchanana Rasa* in different concentrations like 12.5 µg/ml, 25 µg/ml, 50 µg/ml, and 100 µg/ml.

Table 1: Represents table of percent reduction of DPPH after the treatment of *Panchanana Rasa* in different concentrations like 12.5 µg/ml, 25 µg/ml, 50 µg/ml, and 100 µg/ml.

Sample	Reading at 570 nm	% reduction in DPPH radicle
Methanol (0.04mg/ml)	0.89	**0**
Ascorbic acid (10mg/ml)	0.56	**37**
***P. Rasa*12.5ug/ml**	0.33	**63**
***P. Rasa* 25ug/ml**	0.25	**72**
***P. Rasa* 50ug/ml**	0.27	**70**
***P. Rasa* 100ug/ml**	0.29	**67**

Results of inflammation induced in H9C2 (2 – 1) cells using LPS:

Figure 3 illustrates the detailed effects of varying concentrations of Lipopolysaccharide (LPS) on H9C2 (2 -1) cells, with the primary goal of identifying the optimal dosage to induce inflammation. Five different combinations of LPS like 0.6µg/ml, 1.25 µg/ml, 2.5µg/ml, 5µg/ml and 10µg/ml were tested to observe their impact on cell proliferation and cytotoxicity.

Photo micrographic images (Figure 3 i) show the change in proliferation of H9C2 (2 -1) cells after 48 hours of induction with LPS. It was observed that at the highest concentration (10µg/ml), there was a notable increase in

H9C2 (2 -1) cell proliferation compared to the control group ($P > 0.05$ vs. Control), indicating that this dosage stimulated cell growth without inducing significant cytotoxic effects. However, there was no notable increase in cell proliferation at rest of the LPS concentration with no cytotoxic effects as well.

Similarly, these results were confirmed through a cell proliferation assay (Figure 3 ii), where H9C2 (2 -1) cells treated with the different doses of LPS exhibited increment in cell viability and proliferation compared to the control by 67%. The proliferation assay quantitatively supported the microscopic observations, showing that only the highest dose (10µg/ml) of LPS effectively increased cell proliferation without significant cytotoxicity.

Based on these findings, a concentration of 10µg/ml of LPS was selected as the optimal dose for the model system. This concentration allowed for a balance between immune stimulation and cell viability, making it ideal for further research into the immunomodulatory activity of *Panchanana Rasa*. [65,66]

i)

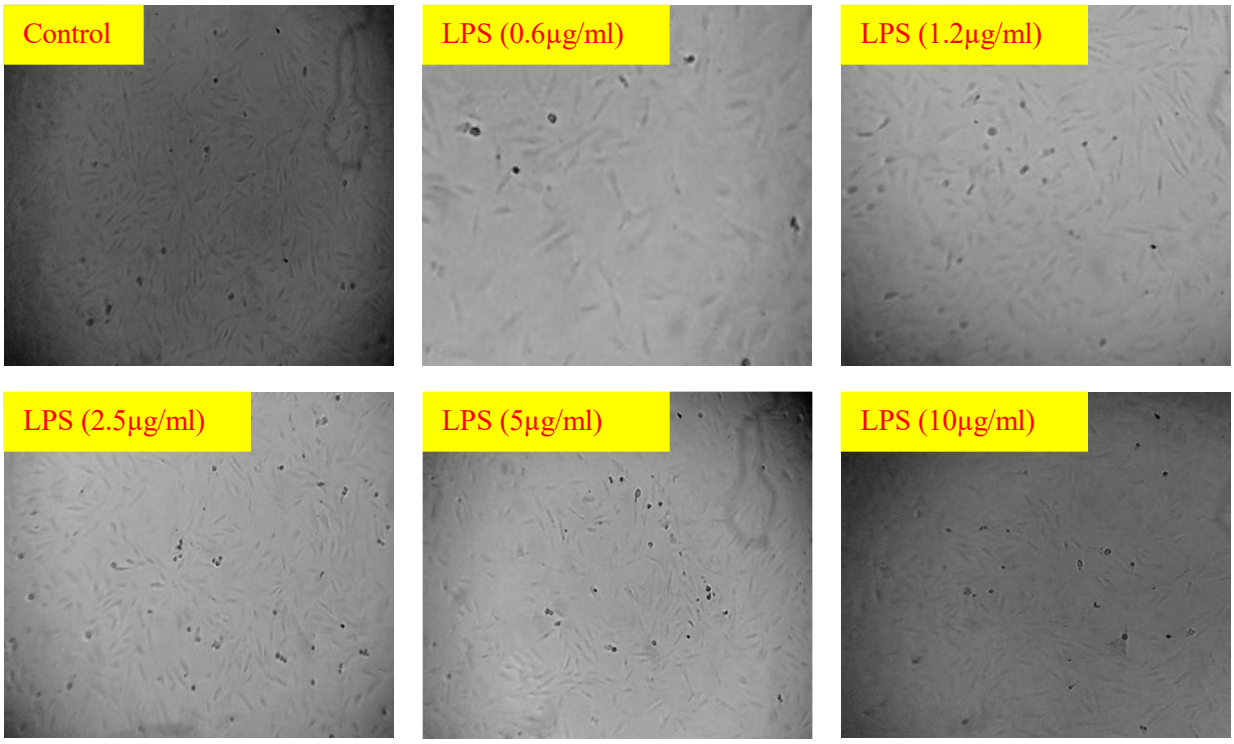
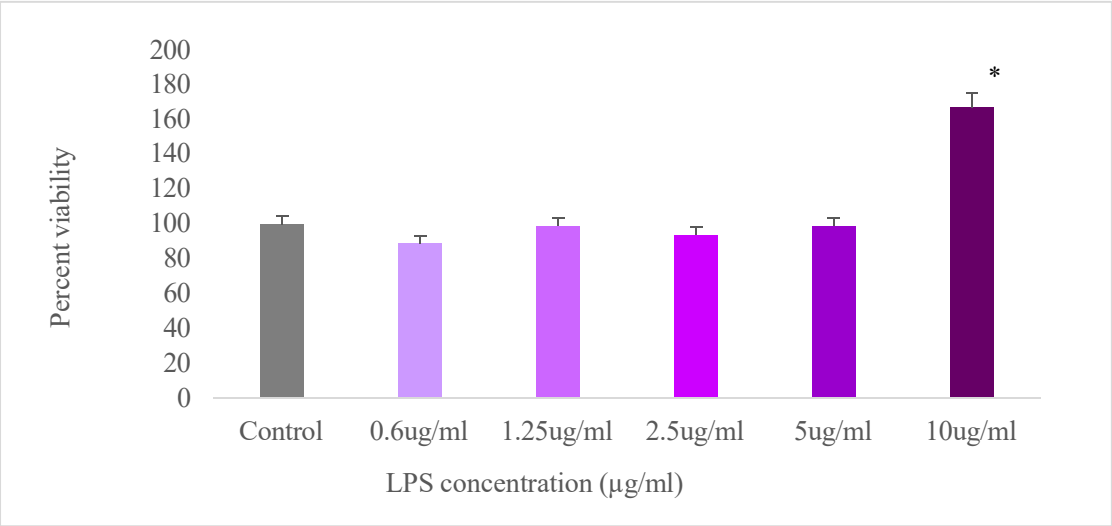


Figure 3: llustrates the effect of Lipopolysaccharide (LPS) H9C2 (2 -1) cells using three different concentrations of LPS like 0.6µg/ml, 1.2µg/ml, 2.5µg/ml, 5µg/ml and 10µg/ml. The effects are depicted in two formats: i) at the microscopic level; and ii) at the viability (proliferation) level.



DISCUSSION:

Panchanana Rasa is a *Sagandha Niragni Murchanna* of *Parada* with *Bhavna* of *Yashtimadhu Kwatha*, *Amalaki Swarasa, Draksha Kwatha* and *Kharjura Kwatha*. It is *Khalviya Rasayana* mentioned in *Hridroga Prakaran* of *Bhaishajyaratnavali.* Although there were many formulations in Ayurveda like *Hridayarnav Rasa*, *Brihat Vaatchintamani*, *Prabhakar Vati*, *Arjunarishta* which are commonly used in treatment of cardiac disorders, a little attention has been paid to *Panchanana Rasa*, a herbomineral formulation in the area of Pharmaceutical and In vitro study. So as to explore this drug Pharmaceutico-analytically with evaluation of its Anti-inflammatory activity, this study was carried out.

In this study, we have discussed -

1.Pharmaceutical Study

2.Analytical study:

3. Experimental Study:

1. Pharmaceutical Study:

Purification of *Parada* (Mercury) is a vital process before using it in *Rasayoga.* Total quantity of *Parada* was taken 500 g and Obtained was 480 g. The loss observed was 20 g. The reason behind loss was spitting of material in *Khalvayantra* during *Prakshalan* of *Parada. Parada* obtained was shiny, bright and mirror like white after trituration in *Rasona Kalka*. Garlic's sulphur compounds are thought to have chelating properties, which means they can help bind to heavy metals like Mercury & facilitate their removal from the body. This chelation process is considered beneficial in reducing the toxic effects of heavy metal poisoning. After purification of *Gandhaka,* some loss was observed due to removal of various impurities, far soluble and water soluble substances. After *Shodhana*, the Sulphur changes from a crystalline to an amorphous nature. This transformation is attributed to the repeated heating, melting, and sudden cooling process.

Kajjali is prepared by taking *Shuddha Parada* 200 g and *Shuddha Gandhaka* 200 g. *Kajjali* was triturated for 15 days to achieve specific *Siddhi Lakshanas* like *Varitartva*, *Nishchandratva* and *Rekhapurnatva*. It is observed

that slow grinding with specific pressure by pestle makes *Kajjali* fine and soft. The loss observed was due to dusting of *Kajjali*. *Kajjali* is believed to have the potential to cure various diseases when used properly in combination with other metals or herbs.

Prepared *Kajjali* was triturated with *Yashtimadhu Kwatha, Amalaki Swaras, Draksha Kwatha and Kharjura Kwatha* each was given per day respectively. *Mardana* was continued till the mixture got dried up. After each *Bhavna Kajjali* becomes sticky with greyish black in colour. *Yashtimadhu* has potent anti-inflammatory properties which has impregnated during *Bhavana* process. Similarly, *Amalaki* contains Emblicanin-A and B useful in reducing the oxidative stress brought on by ischemia-reperfusion in rat cardiac tissue as per previous studies. *Amalaki* fruits are cardioprotective in nature. *Draksha* contains Resveratrol which is cardioprotective and Anti-inflammatory. Preclinical studies have shown that the date fruits possess free radical scavenging, antioxidant, antimutagenic, antimicrobial and Anti-inflammatory activity. In *Bhavana* ,biological changes include reduced particle size ,induction of trace elements , improved therapeutic efficacy (due to formation of desired compounds during the process of *Bhavana*), transfer of organic components of the liquid media to the drug material.

2. Analytical Study:

Prepared drug was analyzed through organoleptic and physiochemical parameters to assess its quality and safety. Instrumental analysis like XRD and SEM was done to evaluate its chemical composition, particle size in order to assess dissolution of drug and fast absorption. pH of *Panchanana Rasa* is 4 indicating its acidic nature. It is due to its *Bhavna Dravya* i.e. *Amalaki* and *Draksha.* It enhances bioavailability of drug when it enters in the GI tract. The LOD of *Panchanana Rasa* was 3.18 % w/w indicating its organic substances as its ingredients which absorb moisture. Total ash value was found 3.23 % w/w because of its organic substances as its ingredients. Acid insoluble Ash value was 1.70% which facilitates the easy absorption of drug and water soluble ash value was 0.70 %w/w. X Ray Diffraction was done for structural analysis. Phase and structure of the compound is studied after comparing the identified 2 theta value with standard 2 theta value. XRD of *Panchanana Rasa* suggest that it contains compound Hg1S1(Cinnabar)as cubic structure and S8 Sulphur with orthorhombic structure. The SEM study of *Panchanana Rasa* showed smallest particle size was ranging between 1 mm at 100X magnification and 50 µm at 2000X magnification. Particle size is one of the factors which will affect the dissolution and absorption of the drug. Particle size and surface area are inversely proportional to each other, as particle size decreases surface area increases. This leads to an increase in the dissolution and rapid absorption.

3. Experimental Study:

In experimental study, we investigated the cytotoxic effect of *Panchanana Rasa* in H9C2 (2-1) cell line, optimization of the LPS concentration to induce inflammation and Antioxiadant activity of *Panchanana Rasa***.** The LC50 value of H9C2 (2-1) was found to be 1106.1µg/ml, which is extremely high and thus it can be concluded that the drug is safe to use for further therapeutic purposes. It suggests that *Panchanana Rasa* will be safe to use in management of Myocarditis in further clinical study. The cell viability remained stable across all tested concentrations suggesting its safety in further experiment. The proliferation assay quantitatively supported the microscopic observations, showing that only the highest dose (10µg/ml) of LPS effectively increased cell proliferation without significant cytotoxicity. In DPPH Assay, *Panchanana Rasa* has potent antioxidant

properties, with its maximum effectiveness observed between 25 µg/ml and 50 µg/ml. Oxidative stress has been main cause of origin of cardiovascular diseases . Due to its anti-oxidant activity, *Panchanana Rasa* will be effective cardioprotective drug for further clinical study. After assessment of XTT assay and DPPH assay for free radicle scavenging, *Panchanana Rasa* can possibly act as anti-inflammatory drug for further immune-modulatory activity.

CONCLUSION:

Based on above findings of pharmaceutical observations, all the parameters were fulfilled according to standard operative procedure. In experimental study, on evaluating cytotoxic effect of *Panchanana Rasa* through XTT assay it was found that *Panchanana Rasa* do not cause toxic effect on H9C2 cell and PBMC cell. We assessed the results through using XTT assay and calculated the LC50 values for *Panchanana Rasa*. In DPPH Assay *Panchanana Rasa* found to be potent Anti-oxidant than Ascorbic acid. After assessment of XTT assay and DPPH assay for free radicle scavenging, *Panchanana Rasa* can possibly act as anti-inflammatory drug for further immune-modulatory activity. Although further studies of immunomodulatory activity of *Panchanana Rasa* with animal model is recommended.

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