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Research Article

A Pharmaceutical and Experimental study of Hepatoprotective activity of *Haridradi Ghrut* in CCl4 induced hepatotoxicity in albino mice

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ABSTRACT:

The Aushadha is like an instrumental aid to a physician. Hence it is placed next to the physician in the Chatushpada of treatment. In today's world liver diseases are among the major health problem because of bad eating habits i.e. junk food, contaminated water, alcohol consumption and harmful drug consumption. Considering the importance of liver and the rate of liver disease it is necessary to conduct research on Ayurvedic formulations to cure liver disorders. In present study one such formulation Haridradighrut mentioned in Charaka Samhita was selected, as the ingredients of Haridradi ghrut has been proved hepatoprotective in earlier studies. The study was conducted to establish pharmaceutical parameters, the physico- chemical parameters and to evaluate it the hepatoprotective activity of Haridradi ghrut. Materials and Method- The preparation of Haridradighrut was carried out under 2 phases- Ghrut Murcchana and Haridradighrut preparation. Experimental study- Standard Drug: - The reference standard drug used for hepatoprotective evaluation is silymarin. Toxicant Drug: Carbon tetrachloride was used for inducing hepatotoxicity in mice. Haridradi ghrut: - Haridradi ghrut was prepared as per textual reference and used for the activity. IAEC approved the protocol for this study. The pharmaceutical study proved that it can be taken as a standard procedure for the preparation of Haridradighrut. Haridradighrut was proved to be effective against CCl4 induced hepatotoxicity in albino mice. The details of the study are given in the paper.

KEY WORDS: Sneha Kalpana, Haridradi Ghrut, Hepatoprotective, Murchchana

INTRODUCTION:

The entire science of Ayurveda has been framed upon 'Trisutras'(Hetu, Linga and Aushadha)[1]. Among them Aushadha is most important. The Aushadha is like an instrumental aid to a physician. Hence it is placed next to the physician in the Chatushpada of treatment [1.]. As the objective of treating the patient can only be attained by the use of drug (drvaya).

Many drugs are used by the Ayurvedic practitioners. These may be Herbal, Herbomineral or Metallic. So the Aushadha Nirman Shastra is divided into two groups-

1. Rasashastra – Drugs prepared predominantly from metals and minerals.
2. Bhaishajyakalpana – Drugs prepared predominantly from herbs.

The science which deals with the process of preparation of single or compound formulation from herbs is known as Bhaishajyakalpana.

Bhaishajyakalpana consists of Basic formulations i.e. Panchavidha Kashaya Kalpana. And other secondary formulations like Vati, Avaleha, Sneha and Sandhan Kalpana etc.The secondary formulations may be established to increase the shelf life, improve palatability and enhance the drug action. Among the formulations Sneha kalpana is one of the most commonly used formulations by the Vaidyas. Sneha kalpana is prepared by using medias made up of lipids (ghrut and tail), water or decoctions and the paste of raw drug.

Now a days practitioners depends on pharmaceutical industry for drugs so there is need for quality assurance of Ayurvedic formulations. For this purpose, there is need to develop standard parameters for every ayurvedic drug which will ensure its quality. And it is possible by conducting pharmaceutical and analytical study of Ayurvedic formulations.

Liver is the largest and vital organ of the body. It performs many different functions like – filtration and storage of blood, metabolism (of carbohydrates,

proteins, fats, hormones, and foreign chemicals), formation of bile, formation of coagulation factors. In case of liver abnormalities it becomes evident that many of the functions gets disturbed simultaneously as they interrelate with one other. [2]

In today's world liver diseases are among the major health problem because of bad eating habits i.e. junk food, contaminated water, alcohol consumption and harmful drug consumption. According to the latest WHO data published in April 2011 India ranks 27th in liver disease and death has reached 2.31% or 208, 185 of total deaths.[3]

As per Ayurveda, Yakrut (liver) is the moolsthan of Raktavaha srotas. Rakta dhatu utpatti takes place in Yakrut. The main role of rakta dhatu is 'jeevan'i.e Pranadharanam.[4] Considering the importance of liver and the rate of liver disease it is necessary to have some remedy to cure liver disorders.

In present study one such formulation Haridradighrut mentioned in Charaka Samhita was selected, as the ingredients of Haridradi ghrut has been proved hepatoprotective in earlier studies. So the study was carried out with a Research question kept in mind, Does Haridradi ghrut shows Hepatoprotective activity in CCl4 induced hepatotoxicity in albino mice? So Haridradi ghrut mentioned by Acharya Charaka was prepared and its pharmaceutical study and Heptoprotective activity was carried out to rule out its efficacy.

Importance of Present Study- The study was conducted to establish pharmaceutical parameters, the physico- chemical parameters and to evaluate it the hepatoprotective activity of Haridradi ghrut.

Aim and Objectives:

- To prepare Haridradi ghrut by classical method.
- To establish the pharmaceutical parameters of Haridradi ghrut.
- To find out the Hepatoprotective activity of Haridradi ghrut by experimental study.
- To observe adverse effect if any.

Study Protocol:-

1. Conceptual Study
2. Pharmaceutical Study
3. Experimental Study

1. Conceptual study:

Conceptual study means understanding of something that is necessary to attain before understanding how it is used or applied. So a detailed study of following has been carried out using available Ayurvedic literature, modern perspectives and journals-

a. Concept of Sneha kalpana- Sneha Kalpana, Ingredients and their ratio, Procedure of sneha Kalpana, Sneha siddhi lakshana and Pariksha, Sneha paka and its uses, Murcchana and its use
b. Drug review- Drug review has been done to explain the therapeutic properties of the ingredients i.e. contents of Haridradighrut, Mahish ghrut, mahish kshir, murcchna dravya. Pharmacognostical study- Detailed morphological, macroscopic and microscopic study of drugs was carried out.
c. Disease review - The detailed study of Ayurvedic and modern aspect on kamala and liver disorders has been done.
d. Experimental study review:- The review of literature on Hepatoprotective activity, Hepatotoxic drugs (carbon tetra chloride) and silymarin was taken,

2. Pharmaceutical study:

In these study details of manufacturing process of Haridradi ghrut was observed.

Pharmaceutical study includes the collection of raw materials, selection of instruments, preparation of kalka for Ghrut murcchana and for Haridradi ghrut and specific observations like temperature pattern, duration and specific findings. All these findings were noted during process and of final product.

3. Experimental study:

The experimental study was conducted to evaluate the Hepatoprotective activity of Haridradighrut in mice. Haematological parameters and histopathological study were also performed and analyzed.

MATERIALS AND METHODS:

Pharmaceutical study

The preparation of Haridradighrut was carried out under 2 phases-

Ghrut Murcchana
Haridradighrut preparation

1. Name of the practical – Preparation of Ghrut Murcchana-

Reference- Bhaishajyaratnavali – jwara chikitsa (5/1285) [5]

Equipments– Heating device- Gas burner with LPG cylinder, Vessels - Stainless steel vessel - Diameter - Outer - 32 cm, Inner - 29.4 cm Depth - 19.5 cm Weight - 1.145 kg Cotton cloth, strainer, Measuring cylinder, Stainless steel ladle, Thermometer.

Procedure-

All the kalka dravyas were made into powder form then kalka was prepared by adding water. Mahish ghrut was measured and taken in a stainless steel vessel and heated over mandagni till moisture gets completely evaporated at that stage temperature was around 144°C. Heat was stopped and then kalka was added in the ghruta when its temperature decreased, after that water was added and again heat was applied. During the process continuous stirring was carried out. Heating was carried out till sneha siddhi lakshana were appeared then the vessel was taken out from fire and kept for cooling. After that Murcchit ghrut was filtered and stored in a glass jar container.

Table No. 1: Showing list of ingredients for Ghrut Murcchana

Ingredients	Quantity
Mahish ghrut	1280 ml
Kalka dravya	
Haritaki	80 gm
Bibhitaka	80 gm
Amalaki	80 gm
Haridra	80 gm
Matulunga swaras	80 gm
Musta	80 gm
Water	5120 ml

Precautions –

- During this process continuous stirring was carried out to protect the burning of kalka especially at the last stage.
- The big sized vessel was taken so as to avoid the loss of ghrut during bumping.
- The temperature was maintained.

2. Name of the practical – Preparation of Haridradi Ghrut-

Reference – Charak samhita Chikitsasthana 16/53 [6]

हरिद्रात्रिफलानिम्बबलामधुकसधितम् ।

सक्षीरमाहिषसर्पिः कामलाहरमुत्तमम् ॥ - च. चि. 16/ 53

Equipments – Heating device- Gas burner with LPG cylinder Vessels - Stainless steel vessel

Diameter - Outer - 50 cm, Inner - 46 cm Depth - 24 cm Weight – 4.698 kg Cotton cloth, strainer, Measuring cylinder, Stainless steel ladle, Thermometer.

Table No. 2: Showing list of ingredients for Haridradi Ghrut

Ingredients	Quantity
Murcchit Mahish ghrut	900 ml
Kalka	
Haridra	16 gm
Haritaki	16 gm
Bibhitaka	16 gm
Amalaki	16 gm
Nimb	16 gm
Bala	16 gm
Yashtimadhu	16 gm
Mahish Ksheer	3600 ml
Water	3600 ml

Procedure-

All kalka dravyas were made into powder form then kalka was prepared by adding water. Murcchit Mahish ghrut was taken in a stainless steel vessel and heated over mandagni till moisture content gets completely evaporated then heating was stopped and kept for cooling. When ghrut was luke warm kalka was added to the ghrut followed by water and milk again heat was applied and continuous stirring was done. Heating process was carried out till sneha siddhi lakshana appeared then the vessel was taken out from fire and ghrut was filtered. After cooling Haridradi ghrut was stored in a glass jar container.

Precautions –

- Before adding milk to the ghrut temperature was maintained around 40^{o}c in both liquid media.
- Continuous stirring was carried out to protect the burning of kalka especially during the last stage.
- The big sized vessel was taken so as to avoid the loss of ghrut during bumping.
- The temperature was maintained.

Experimental study

Drug:

- Haridradi ghrut: Haridradi ghrut was prepared as per textual reference [5] and used for the activity.
- Standard Drug: The reference standard drug used for hepatoprotective evaluation is silymarin. It was purchased from the market with the trade name Silybon.
- Toxicant Drug: - Carbon tetrachloride was used for inducing hepatotoxicity in mice.

Selection of Animal: - Healthy young Swiss Albino mice between 1 and 2 months of age (male) weighing 20-25 g were randomly selected and divided into the control and treatment groups. 24 mice were acclimatized for 5 days prior to dosing. During this period, animals were observed daily for clinical signs.

Housing: Animals were maintained at 25± 2^{0}C and relative humidity of 45 to 55% & under standard environmental conditions (12 h light and 12 h dark cycle). The food and water were provided ad libitum.

IAEC Approval: IAEC protocol for this study was approved.

Design of Experiment:-

Four groups viz. vehicle control group, Hepatotoxicity (negative) control, Silymarin control, Haridradighrut were used to study the Hepatoprotective effect of Haridradighrut in CCl_4 induced hepatotoxicity in mice. Each group had six animals. All animals from each treatment group received treatment as per mentioned in Table No.1 for 07 days and at the end of 7th day hepatotoxicity was induced in all the animals (except the vehicle control group). At the end of study the blood has been collected from retro –orbital plexus of all animals and finally all animals were sacrificed, liver was isolated examined for the histopathological examinations. The dose for mice was calculated extrapolating the human therapeutic dose (HTD).

Table No.3: Treatment Protocol for Hepatoprotective Activity

Gr. No.	Group Description	Treatment
I	Vehicle/Normal Control	Received distilled water for 07 days
II	Hepatotoxicity (Negative) Control	Received distilled water for 07 days
III	Silymarin Control	Received suspension of silymarin tablet in water for 07 days
IV	Haridradighrut Treated	Received emulsion of Haridradighrut in distilled water for 07 days

Dose fixation:

Test drug: Haridradighrut: 40 gm/day

i.e. Human Equivalent Dose (HED mg/kg) = 666.67 mg/kg

Animal Dose= 666.67/0.081 = 8230.45 mg/kg

Silymarin: 100 mg/kg

Carbon tetrachloride: 0.5 mg/kg in olive oil

Administration of Doses: The entire test drugs were suspended/ emulsified in distilled water and administered using oral gavage. The normal control and the Hepatotoxicity (Negative) control groups were given distilled water for 07 days starting from day 1.

Induction of Hepatotoxicity: The Hepatotoxicity was induced in all mice except vehicle control group at the end of study by an intra-peritoneal injection of CCl_4 (Carbon tetra chloride-LobaChem) (0.5 mg/kg in olive oil).

Parameters under Study:

1. Treatment related clinical signs and mortality: All the animals were observed for treatment related clinical signs and mortality if any
2. Body weight: Weekly body weight of all animals were done and used for dose calculation.
3. Biochemical estimations: At the end of study Biochemical Estimation was done consisting of parameters like, SGPT, SGOT, ALP, Total protein, Total Bilirubin.
4. Gross Pathology & Histopathology of Liver

All animals were sacrificed by ether anesthesia. Soft tissues like liver were removed from the body, wash with distilled water and NS. Preserve in 10% Formalin buffer solution, Tissue samples were prepared for light microscopy using standard procedures. The microscopic observations were done for hepatocytes damage area, and inflammatory cells.

OBSERVATIONS AND RESULTS:

A. Pharmaceutical study

During Ghrut Murchchana- After 1hr of heating the colour of ghrut changed to greenish yellow colour. A homogenous mixture was formed after 2hrs and colour gets darken. Later after 4 hrs the layer the layer of ghrut and kalka appeared seperate. Pleasant smell of mixture present during the heating process. During boiling process the ghrut started bumping outside the vessel. After 7 hrs kalka started sticking to the vessel and difficulty in stirring was felt.

Table No. 4: Temperature during preparation of ghrut murcchana in different stages

Sr. No.	Observation	Temperature (°C)
1	Temperature observed when ghrut becomes moisture free	144 °C
2	Temperature when kalka was added in ghrut	64 °C
3	Temperature when water was added	40 °C
4	Temperature observed after 5 min after starting heat	50 °C
5	Temperature observed after 30 min	70 °C
6	Maximum Temperature obtained during ghrut murrchana	96 °C
7	Average Temperature during ghrut murcchana	94 °C
8	Temperature observed at the time of Phenashanti stage	90 °C
9	Temperature observed at the time of mridupaka stage	90 °C
10	Temperature observed at the time of madhyampaka stage	90 °C
11	Temperature at the time of filtration	70 °C

Table No. 5: Duration of ghrut murcchana in different stages

Sr. No.	Observations	Duration
1	Total duration to obtain moisture free condition	10 min
2	Kalka added	20 min
3	Water added	22 min
4	Duration for phenashanti	7hr 15 min
5	Duration for Mridupaka	7hr 30 min
6	Duration for madhyampaka	7hr 55 min
7	Total time required for Ghrut murcchana	8 hr

Table No. 6: Completion test during preparation of ghrut murcchana

Sr. No	Name of test	Media	
		Kalka	Ghruta
1.	Fire test	+ve	+ve
2.	Ishta gandha varna rasotpatti	NA	+ve
3.	Phenashanti	NA	+ve
4.	Shabdhinatva	NA	+ve
5.	Varti	+ve	NA

Table No. 7: Result obtained during preparation of Ghrut Murcchana

Sr. No.	Results	
1.	Initial quantity of ghrut	1280ml
2.	Final quantity of ghrut obtained	1120ml
3.	Total loss of ghrut in ml	160ml
4.	Loss of ghrut in %	12.5%
5.	Initial quantity of kalka	480gm
6.	Weight of kalka after filtration	771gm
7.	Colour of Kalka	Dark green

Table No. 8: Organoleptic characters during murcchana

Organoleptic test	Before murcchana	After murcchana
Sparsha	Sticky/Greasy	Sticky/Greasy
Rupa	Creamish white	Yellow
Rasa	Not specific	
Gandha	Characteristic	Pleasant/ characteristic

During Preparation of Haridradi Ghrut-

After 1 hr the colour of ghrut changed from yellow to dark yellow. Separate layer of Ghrut and kalka appeared. Dark yellow colour fat globules on the top layer of mixture appeared. Homogenous mixture formed after 2 hrs of heating. Ghrut started separating after 7hrs of heating and ghrut started bumping outside the vessel. After 9 hrs mawa was formed (consistency gets thickened). Kalka started sticking at the bottom of the vessel and difficulty in stirring was felt.

Table No. 9: Temperature during preparation of Haridradi Ghrut in different stages

Sr. No.	Observation	Temperature(°C)
1	Temperature observed when ghrut becomes moisture free	46°C
2	Temperature when kalka was added in ghrut	64°C
3	Temperature when water was added	40°C
4	Temperature when milk was added	38°C
5	Temperature observed after 5 min after starting heat	45°C
6	Temperature observed after 30 min	62°C
7	Maximum Temperature obtained during ghrut paka	98 °C
8	Average Temperature during ghrut murcchana	94 °C
9	Homogenous mixture of ghrut and kalka	80 °C
10	When separation of ghrut from kalka occurs	88 °C
11	When mridupaka was observed	90 °C
12	When madhyam paka was observed	94 °C
13	Temperature at the time of filtration	60 °C

Table No. 10: Duration and observations during preparation of Haridradi Ghrut

Sr. No.	Observations	Duration
1	Total duration to obtain moisture free condition	10 min
2	Kalka added	22 min
3	Water added	25 min
4	Ksheer added	28 min
5	Homogenous mixture	2 hrs
6	Separation stage observed	8hr40min
7	Duration for mridupaka	9 hr
8	Duration for madhyampaka	11 hr 30 min
9	Total hrs for ghrut paka	11 hr 30 min
10	Total days required for ghrut paka	2 days

Table No. 11: Completion test during preparation of Haridradi Ghrut

Sr. No.	Name of test	Media	
		Kalka	Ghruta
1.	Fire test	+ve	+ve
2.	Ishta gandha varna rasotpatti	NA	+ve
3.	Phenashanti	NA	+ve
4.	Shabdhinatva	NA	+ve
5.	Varti	+ve	NA

Table No. 12: Result obtained during preparation of Haridradi Ghrut

1.	Initial quantity of Murcchit Mahish ghruta	900 ml
2.	Final quantity of Haridradighrut obtained	670 ml
3.	Total loss of ghrut in ml	230 ml
4.	Loss of ghrut in %	25.5%
5.	Initial quantity of kalka	112 gm
6.	Weight of kalka after filteration	1.150 gm
7.	Colour of Kalka	Brown

Table No. 13: Organoleptic characters during preparation of Haridradi Ghrut

Organoleptic test	Observation
Sparsha	Sticky/ greasy
Rupa	Yellow
Rasa	Slight sweet
Gandha	Pleasant/characteristic

B. Experimental study

Table No.14: Observations for Body Weight

Sr. No	Body Weight	Vehicle Control	Hepatotoxicity (Negative) Control	Silymarin Control	Haridradighrut Treated
1.	On 1st Day	20.173 ± 0.2521	20.278 ± 0.4238	21.162 ± 0.6411	21.242 ± 0.7285
2.	On 7th Day	25.880 ± 1.583	28.625 ± 0.7874	26.400 ± 0.5927	28.318 ± 1.172

Table No. 15: Observations for Biochemical Parameters

Sr. No	Group	SGPT	SGOT
1.	Vehicle Control	16.667+1.116	42.500 + 1.232
2.	Hepatotoxicity (Negative) Control	152.67+8.123##	164.33 + 4.558##
3.	Silymarin (standard) Control	8.833+ 1.108**	49.667 + 2.985**
4.	Haridradighrut Treated	30.333+ 6.736**	72.833 + 4.564**

Table No. 16: Observations for Biochemical Parameters

Sr. No.	Group	ALP	Total Protein	Total Bilirubin
1.	Vehicle Control	169.1 +11.926	255.02 + 13.256	1.033 + 0.2216
2.	Hepatotoxicity (Negative) Control	234.17+9.700##	213.50 + 4.970##	3.150 + 0.2778#
3.	Silymarin (standard) Control	236.33+12.917ns	233.72 + 5.526ns	5.083 + 1.013*
4.	Haridradi Ghrut Treated	240.33+12.366 ns	230.88 + 8.213ns	2.783 + 0.3572 ns

Results were presented as mean ± SEM. (n = 6) One-Way Analysis of Variance (ANOVA) followed Dunnett Multiple Comparisons Test. # P< 0.05, ## P< 0.01,#nsP>0.05 when compared with Vehicle Control; * P<0.05, ** P<0.01, ns P>0.05 when compared with Hepatotoxicity (Negative) Control.

Table No. 17: Comparative Data of Liver Histopathology Observations (Staining with H & E (20x))

Sr. No.	Vehicle Control	Hepatoxicity (Negative) Control	Silymarin (Standard) Control	Haridradi Ghrut Treated
1.	Normal hepatocytes. Nothing Abnormal Detected	Minimal focal centrilobular hepatocyte degeneration. There is marked hypertrophy of hepatocytes and MNC infiltration	Mild to moderate hepatocyte degeneration and hypertrophied hepatocytes and hypertrophy of hepatocytes is slightly reduced, however the architecture is disturbed Minimal MNC infiltration	Reduced hepatocyte degeneration, minimal focal hepatocyte degeneration and hypertrophy of hepatocytes and There is minimal MNC infiltration

DISCUSSION:

Pharmaceutical study:

Ghrut murrcchana was carried out by taking an initial quantity of 1280ml (2 prastha) of ghrut and the drugs required for kalka were taken with the respective ratio of ingredients mentioned in Bhaishjyaratnavali (each dravya 2 pala). Appropriate amount of water was added to the powder and kalka was prepared.

Mahish ghrut was taken in stainless steel vessel and mild heating was done for 10 min till it became moisture free, cracking sound stopped at that time temperature was 144^{0}C. Then the heating was stopped and ghrut was kept for slight cooling when temperature decreased to 64^{0}C kalka was carefully added to it. Immediately after adding kalka cracking sound and froth appeared in the mixture due to the water content in kalka, 4 times water was added in the mixture and subjected to heat. Phenashanti stage was observed after 7hrs 15 mins at that time temperature was 90^{0}C .At this stage moisture content was very less which was examined by varti parikshana and agniparikshana. Mridupaka stage was observed after 7 hrs 30 mins and Madhyampaka stage was observed after 7hrs 55 mins, at this stage kalka became completely moisture free and the temperature was 90^{0}C at both the stages .The madhyampaka stage was confirmed as kalka converted to soft varti and the absence of cracking sound when subjected to fire (agniparikshan) indicating the absence of moisture. The average temperature maintained throughout the process was 940C. The total duration required for the completion of snehapaka of ghrut murcchana was 8 hrs. Total duration for the completion of senha paka of ghrut murcchana was 8 hrs.

Final quantity of ghrut obtained was 1120ml. Total loss in percent was 12.5%.It was due to the bumping of ghrut outside the vessel and absorption of sneha by kalka. Weight of kalka after filteration increased as the kalka dravyas are ruksha in nature and have capacity to absorb the ghrut (Sneha). The colour of kalka was dark green.

The colour of obtained Murcchit Mahish ghrut was dark yellow because of the colouring constituents of Haridra. It had a characteristic pleasant smell of kalka dravyas and the taste of ghrut was astringent (kashaya) because of Triphala, Musta and Haridra.

For the preparation of Haridradighrut, 900ml of Murcchit mahishghrut was taken as sneha dravya and kalka was taken 1/8th part of sneha(112gm) as when snehapaka has to be carried out with ksheer kalka dravya should be taken1/8th part of Sneha as mentioned in Sharangdhara Samhita. The quantity of Mahish ksheer and water was taken 4 times that of ghrut (3600 ml each). Ksheer was first boiled and kept for cooling. Then murrchit ghrut was taken in vessel and kept for heating. After 10 min ghrut became moisture free (cracking sound stopped) at that time temperature was 146^{o}C.Kalka was added in ghrut at 64^{o}C, cracking sound and froth appeared soon after adding kalka. Then water was added followed by mahish ksheer. Before adding ksheer in sneha the temperature was below 40oC in both the liquid medias (milk and sneha) to avoid the spoiling of milk. If the temperature in any of the liquid media is more than 50^{o}C then the chances of spoiling of milk increases. Because at higher temperature the protein in milk gets aggregated and temperature variation in both liquid media may lead to sudden aggregation of protein and spoiling of milk.

After 2 hrs of heating homogenous mixture was formed and the separation between kalka and ghrut was noticed after 8hrs 40mins. After 9hrs mridupaka stage appeared and mawa like consistency was formed at this stage temperature was around 90^{o}C .During this stage continuous stirring was done and the kalka was sticking to the bottom of the vessel, so difficulty in stirring was felt. After 11hrs 30mins Madhyampaka stage was observed the temperature was 90^{o}C .No moisture content was present in kalka .Total duration required for the completion of procedure was 11hrs and 30mins. The heat was subjected intermittently for 2 consecutive days as per

textual reference when sneha paka has to be performed with ksheer heat should be given for 2 days. The average temperature maintained throughout the process was 94° C.

Total quantity of Haridradighrut obtained was 670 ml. loss of ghrut in percentage was 25.5%. Loss was due to the bumping of ghrut from vessel, kalka being ruksha has absorbed some sneha part. The quantity of kalka increased may be due to the protein part of ksheer, colour of kalka was brown.

The colour of Haridradighrut was dark yellow due to the haridra, had a characteristic pleasant smell of kalka dravyas and taste was slight sweet (madhur) due to madhuk.

Experimental study:-

The main objective of experimental study was to find out the Hepatoprotective activity of Haridradighrut in CCl4 induced hepatotoxicity. The standard drug used for the activity was Silymarin . 24 mice weighing 20-25 gm and of 1-2 months age were randomly divided in 4 groups i.e. control group, hepatotoxicity (negative) control , Silymarin(standard) control and Haridradighrut treated. At the end of study the Biochemical estimation was done of parameters like SGPT, SGOT, Alkaline phosphatase, Total bilirubin and total protein .Animals were sacrificed at the end of study and histopathological assessment of liver was done. After the completion of experimental study Haridradighrut was found effective in CCl4 induced Hepatotoxicity in Albino Mice.

CCl4 is known to cause hepatic damage manifested in marked elevation in serum levels of amino transferase enzymes (SGOT and SGPT) especially ALT which is considered the primary and specific marker of liver injury. Accordingly, our results show a significant increase in the biochemical parameters thereby confirming the hepatocellular damage in CCl4 treated mice.

The level of marker enzymes (biochemical parameters) were elevated in CCl4 treated groups as compared to vehicle control group. The level of Total protein was decreased in CCl4 treated group as compared to vehicle control group.

The group treated with Haridradighrut showed significant decrease in the level of SGOT and SGPT as compared to CCl4 treated group. In Silymarin treated group it also showed significant decrease. In case of Alkaline phosphatase both Haridradighrut and Silymarin treated group does not show significant decrease but were equally effective. The Total Protein in both Haridradighrut and Silymarin treated group showed significant result and were equally effective. The Total bilirubin in Haridradighrut showed significant decrease as compared to CCl4 treated group then silymarin. Reduced Hepatocytes degeneration is also seen in Haridradighrut treated group.

CONCLUSION:

The pharmaceutical study proved that it can be taken as a standard procedure for the preparation of Haridradighrut. Haridradighrut was proved to be effective against CCl4 induced hepatotoxicity in albino mice. A significant decrease in the level of marker enzymes SGOT, SGPT and total bilirubin were seen. Reduced hepatocytes degeneration was also seen in Haridradighrut treated group. No adverse effects were seen. But Haridradighrut was not more effective than the standard drug Silymarin.

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