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STUDY OF HEPATOPROTECTIVE ACTIVITY OF DHATRI AVLEHA IN ALBINO MICE

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ABSTRACT

Avaleha is that form of drug delivery system in which absorption starts right from oral cavity due to its mode of administration i.e. by licking. *Avaleha* due to its good palatability, wide therapeutic applicability is used since ancient period. *Avaleha kalpana* is modified form of *panchvidhkashaya kalpana* which make drug material available throughout year. *Acharya Kashyapa* mentioned separate *adhyaya* in *sutrasthana* due to its importance.(1) It has good palatability, high dietic value and also having their own pharmacological activity. It is easily taken by all age group i.e. *bala, yuva, vruddha*. It is *Shadendriyaprasadak* and nourishes all *dhatu* and *Oja*.(2) It is very easy to administer and palatable. *Yakrut* is *moolasthan* of *Raktavahastrotas.Dhatri* is well known *dravya* for its *rasayana* property. *Dhatri* fruit helps to purify *Rasa* and *Raktadhatu* thus supporting the functions of liver. It also strengthens the liver, helping it in eliminating toxins from body. It has antioxidant property and free radical scavengers helping to reduce disease. It is enriched with Vitamin C which have antioxidant activity and help in detoxification of free radical. Vitamin C and Carotenoids can generate vitamin E from Tocopheroxyl radical E, permitting the vitamin E once more to act as an antioxidant. *Dhatriavaleha* is mentioned in *charaksamhita* in *Pandurogchikitsa* which is mentioned to use in *Pandu, Kamala, Halimak*, these diseases are related to *Raktavahastrotas*. Due to its properties and availability of this formulation *(Dhatriavaleha*), It is selected for hepatoprotective activity against CCl_4 induced hepatotoxicity in albino mice.

Keywords: *Dhatri Avleha*, Hepatoprotective activity, Albino mice

INTRODUCTION

Liver is a vital organ which performs the normal metabolic homeostasis of the body as well as biotransformation, detoxification and excretion of many endogenous and exogenous compounds, including pharmaceutical and environmental chemicals. According to the latest WHO data published in May 2014 Liver Disease Deaths in India reached 2.44% of total deaths. The age adjusted Death Rate is 21.96 per 100000 of population ranks India #61 in the world. In liver diseases hepatitis is major cause of death. Drug induced hepatotoxicity is a major cause of iatrogenic diseases. Most of hepatotoxic chemicals (certain antibiotics, chemotherapeutic agent, carbon tetrachloride, thioacetamide) alcohol consumption and microbes damage liver cells mainly by oxidative stress and lipid peroxidation. In liver diseases there are no early

symptoms and later it may present itself as simple fatigue, anaemia, recurrent jaundice, and swelling in leg or low platelet count. People wouldn't even realise that they have this problem since back, hence these liver diseases are silent killer. There is no specific treatment in modern medicine for liver diseases. Considering prevalence of disease there is increase interest in alternative medicines for treatment of liver diseases.

Refrence of *Dhatri Avleha* is found in *Charak Samhita Chikitsasthana* 16/101-102 used in *Pandu, Kamala, Halimak. Yakrut* is *moolasthan* of *Raktavahastrotas*. *Dhatri* is well known *dravya* for its *rasayana* property. *Dhatri* fruit helps to purify *Rasa* and *Raktadhatu* thus supporting the functions of liver. It also strengthens the liver, helping it in eliminating toxins from body. It has antioxidant property and free radical scavengers helping to reduce disease. It is enriched with Vitamin C which have antioxidant activity and help in detoxification of free radical.Vitamin C and Carotenoids are able to generate vitamin E from Tocopheroxyl radical E, permitting the vitamin E once more to act as an antioxidant, hepatoprotective activity against CCl_4 induced hepatotoxicity in albino mice.[3] Hence experimental study was carried out to prove so.

Aim & Objectives:

- To prepare *Dhatriavaleha.*
- To study hepatoprotective effect of *Dhatri avaleha* in CCl4 induced hepatotoxicity in albino mice.

Material & Method: Reference: *Charak Chikitsasthana* 16/101-102

Raw materials (*Amalaki, mannuka, pippali, shunthi, yashtimadhu. vanshlochana, madhu, Sharkara*) required to prepare *DhatriAvaleha* are collected from reliable source.

Before preparation all raw materials (*amalaki, mannuka, pippali, shunthi, yashtimadhu*) are authenticated in reliable institute and Agmark honey is taken standardization of *Vanshlochana* is done.

Ingredients:

Table 1: Showing Quantity of ingredients taken[4]

Sr no	Contents	Ingredients	Quantity
1	*Drava –dravya*	*Amalaki swarasa*	2050 lit
2	*Madhur dravya*	*Sharkara*	400 gm
3	*Kalka dravya*	*Seedless mannuka kalka*	128 gm
4	*Prakshep dravya*	*Pippali*	128 gm
		Shunthi	16 gm
		Yashtimadhu	16 gm
		Vanshlochan	16 gm
		Madhu	128 gm

1.Preparation of *amalaki swarasa*

Purpose: Preparation of *drava-dravya*

Type of procedure: *Yantranishpiditad* method

Equipment: Knife, mixer, cotton cloth, stainless steel vessel, Measuring cylinder.

Ingredients*: Amalaki fruits*-4 kg

Procedure*: Dhatri Avleha* was prepared according to the process mentioned in Reference: *Charak Chikitsasthana* 16/101-102

Method of preparation:

Amalaki swarasa and sugar was heated and stirred continuously *manuka kalka* was added and *pakalakshana* were observed then fine powder of *prakshepdravya (Pippali, shunthi, Yashtimadhu,vanshlochana*) were added after removing vessel from heat after cooling *madhu* (honey) was added and prepared *avleha* was stored in air tight container.

Precautions: Continuous stirring should be needed throughout procedure; heat should be mild and *prakshep dravya* should be very fine.

***Siddhi Lakshana* observed:** *Siddhi Lakshana* were observed according to the given *lakshanas* in the refrence *CharakChikitsasthana* 16/101-102

Experimental Study: Animal studies have vital role in science development. Experimental studies are important for the development of new drugs. Several experimental models are employed to assess Hepatoprotective activity. Paracetamol, Carbon tetrachloride (CCl_4) and Alcohol induced liver injury in rodents, continues to be the most widely used model. D-galactosamine, Isoniazid, Thioacetamide, Rifampicin etc are the other chemicals employed for inducing liver injury.

In present study Hepatoprotective effect of *Dhari Avaleha* was evaluated in CCl_4 induced hepatotoxicity in albino mice and then it compared with Standard drug silymarine.

1. Methodology:

Selection of Animals:

- Species: Healthy swiss albino mice between 1 and 2 months of age (male)
- Weight: 20-25 g
- These mice were randomly selected and divided into following groups:
  - The control groups.
  - The treatment groups.
- Acclimatization :35 mice will be acclimatized at least for 5 days prior to dosing.
- During this period, animals will be observed daily for clinical signs.

Housing:

- Animals were maintained at 25± 2^0c 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.

Drugs

The drug used for hepatoprotective evaluation are as follows:

- Test drug – *Dhatri Avaleha* used as test drug.
- Standard drug -Silymarin was used as standard for activity. It was purchased from local market with trade name Tab.silybon 70mg.
- Hepatotoxicant drug– Carbon Tetrachloride (CCl_4) was used to induce hepatotoxicity in mice. It was purchased from Loba chem.

Grouping

1) Normal control group
2) Disease control group
3) Standard drug group
4) Test drug group

Experimental design:

- Four groups were used to evaluate hepatoprotective activity in CCl_4 induced hepatotoxicity in albino mice.
- Each group contain six mice.
- All animals from each group received the treatment for seven days as mentioned in table.
- Carbon tetrachloride was induced at the end of 7^{th} day to induce hepatotoxicity in all animals except normal control group.
- Blood sample collected from retri-orbital plexus of all animals at the end of the 7^{th} day.
- Lastly all animals were sacrificed, liver was isolated for histopathological examination.

Dose: The dose of mice was calculated extrapolating the human therapeutic dose (HTD)

1) Test drug (Dhatri Avaleha)

Human dose: 10 gm/day ≈ 10000 mg/day

For Adult Human (60 Kg Weight)

So for converting it to mg/kg = 10000/60 = 1667.67 mg/kg i.e. Human Equivalent Dose (HED)10000/60= 1667.67 mg/kg

For converting it to animal dose:

(HED mg/kg) = Animal Dose (mg/kg) Animal Km/Human Km

Human Km = 37

Animal Km=3

Animal Dose= 166.67/0.081 = 2057.65 mg/kg

Animal Dose= 2057.65 mg/kg

For 20 gm of mice= (2057.65) (20)/1000

= 41.15 mg/day for 20 gm mice

2) Standard drug: Silymarine – 100 mg/kg

3) Carbon tetrachloride- 0.5 mg /kg

Route of Drug Administration

- Distilled water was given to Normal control and disease control group for seven days.
- Suspension of Dhatri Avaleha and silymarine administered orally by using gavage for seven days.

Table 1: Treatment Protocol for Hepatoprotective Activity

Gr. No.	Group Description	Treatment
I	Normal (Vehicle) Control	distilled water was given for 07 days
II	Disease (Negative) Control	distilled water was given for 07 days
III	Standard drug group (Silymarine)	suspension of silymarin tablet in distilled water was given for 07 days (Dose 100 mg/kg)
IV	Test drug (*Dhatri Avaleha*)	suspension of *Dhatri Avaleha* in distilled water was given for 07 days (Dose 10gm/day)

Induction of Hepatotoxicity:

The hepatotoxicity will be induced in all mice except vehicle control group at the end of study by an intraperitonial injection of CCl_4 (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 was 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, Direct Bilirubin from serum.
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 normal saline.
- 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.

Observation and Results

Observations for Biochemical Parameters:

Table 2: Showing observations of biochemical parameters

Sr. No	Group	SGPT	SGOT
1.	Vehicle Control	16.667±1.116	42.500 ± 1.232
2.	Disease (Negative) Control	152.67±8.123$^{\#\#}$	164.33 ± 4.558$^{\#\#}$
3.	Silymarine Control	8.833± 1.108**	49.667 ± 2.985**
4	DhatriAvaleha Treated	53.833± 2.982**	62.833 ± 3.240**

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, #ns P>0.05 when compared with Vehicle Control; * P<0.05, ** P<0.01, ns P>0.05 when compared with Hepatoxicity (Negative) Control.

Table 3: Showing observations of biochemical parameters

Sr. No	Group	ALP	Total Protein	Total Bilirubin
1.	**Vehicle Control**	169.17 ±11.926	255.02 ± 13.256	1.033 ± 0.2216
2.	**Disease (Negative) Control**	234.17 ±9.700$^{\#\#}$	213.50 ± 4.970$^{\#\#}$	3.150 ± 0.2778$^{\#}$
3.	**Silymarine Control**	236.33 ±12.917^{ns}	233.72 ± 5.526^{ns}	5.083 ± 1.013*
4	**DhatriAvaleha Treated**	184.50 ± 8.192*	59.167 ± 6.442**	1.233 ± 0.1406*

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, #ns P>0.05 when compared with Vehicle Control; * P<0.05, ** P<0.01, ns P>0.05 when compared with Hepatoxicity (Negative) Control.

Observations for Histopathology of Livers *(Staining with H & E (20x))*

Table 4: Showing observations of histopathology of liver in vehicle control group

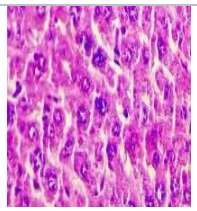
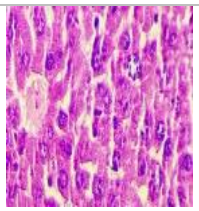
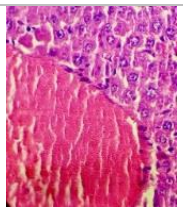
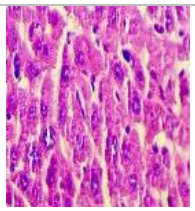
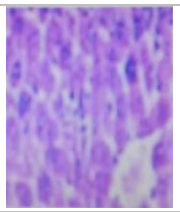
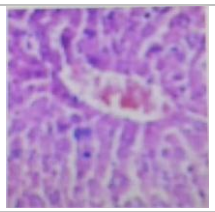
Vehicle control					
VC-1	**VC-2**	**VC-3**	**VC-4**	**VC-5**	**VC-6**
					
Information: Normal hepatocytes. Nothing Abnormal Detected					

Table 5: Showing observations of histopathology of liver in negative control group

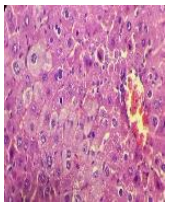
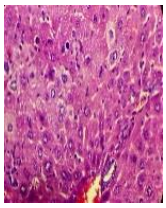
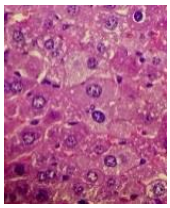
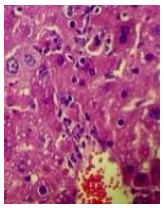
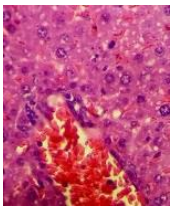
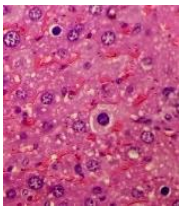
Hepatotoxicity (negative) control					
HNC-1	**HNC-2**	**HNC-3**	**HNC-4**	**HNC-5**	**HNC-6**
					
Information: Minimal focal centrilobular hepatocyte degeneration. There is marked hypertrophy of hepatocytes and MNC infiltration.					

Table 6: Showing observations of histopathology of liver in standard control group

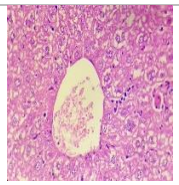
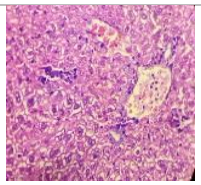
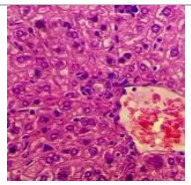
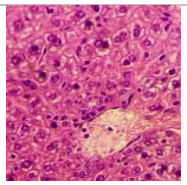
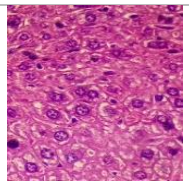
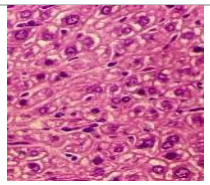
Silymarin(standard) control					
SC-1	**SC-2**	**SC-3**	**SC-4**	**SC-5**	**SC-6**
					
Information: Note mild to moderate hepatocyte degeneration and hypertrophied hepatocytes and hypertrophy of hepatocytes is slightly reduced, however the architecture is disturbed Minimal MNC infiltration.					

Table 7: Showing observations of histopathology of liver in Dhatriavaleha treated group

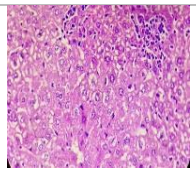
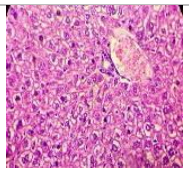
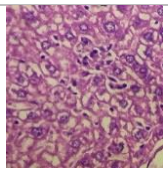
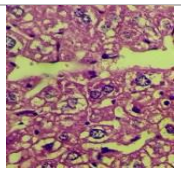
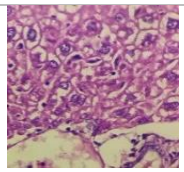
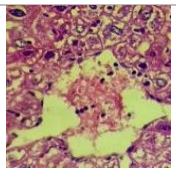
Dhatriavaleha Treated					
DAT-1	**DAT-2**	**DAT-3**	**DAT-4**	**DAT-5**	**DAT-6**
					
Information: Note mild hepatocyte degeneration and hypertrophy of hepatocytes is slightly reduced, however the architecture is disturbed and MNC infiltration.					

Table 8: Showing Comparative Data of Liver Histopathology Observations *(Staining with H & E (20x))*

Sr. No.	Vehicle Control	Hepatoxicity (Negative) Control	Silymarin (standard) Control	*Dhatri Avaleha* Treated
1.	Normal hepatocytes. Nothing Abnormal Detected	Note minimal focal centrilobular hepatocyte degeneration. There is marked hypertrophy of hepatocytes and MNC infiltration	Note mild to moderate hepatocyte degeneration and hypertrophied hepatocytes and hypertrophy of hepatocytes is slightly reduced, however the architecture is disturbed Minimal MNC infiltration	Note mild hepatocyte degeneration and hypertrophy of hepatocytes is slightly reduced, however the architecture is disturbed and MNC infiltration

DISCUSSION

Hepatoprotective Activity: Liver damage is very common since liver has the capacity to detoxicate toxic substances. Hepatoprotective effect was studied against chemicals and drugs induced hepatotoxicity inanimals. Carbon tetrachloride (CCl4), galactosamine, d-galactosamine, thioacetamide, antitubercular drugs, paracetamol, arsenic etc., are used to induce hepatotoxicity in laboratory animals.[5]

Liver injury caused by hepatotoxins, ischaracterised by varying degrees of hepatocyte degeneration and cell death via either apoptosis or necrosis. These drug causes hepatotoxicity by producing reactive oxygen species which cause depletion in tissue thiol, lipidperoxidation, plasma membrane damageMechanism ofhepatotoxicity by various toxins are described in details.Liver injury can be diagnosed by certain biochemical markers like alanine aminotransferase[ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP]and bilirubin. Elevations in serum enzyme levels are taken as therelevant indicators of liver toxicity.[6]

Silymarine is flavonolignan from the seeds of milk thistle has been widely used from ancient times because of its excellent hepatoprotection. Silymarin can neutralise the hepatotoxicity of several agents including ethanol, paracetamol(acetaminophen) and carbon tetrachloride in animal models. Various experimental studies strongly suggest that its hepatoprotective effects are mainly due to free radical
scavenging.

Experimental study[7]: A single drug cannot be effective against all types of severe liver diseases. Effective formulations must be developed using medicinal plants, with proper pharmacological experiments. In this study Hepatoprotective activity of Dhatri avaleha was evaluated in CCl_4 induced hepatotoxicity in albino mice.

Carbon Tetrachloride is metabolized by cytochrome P-450in endoplasmic reticulum and mitochondria with the formation of CCl3O-, a reactive oxidative free radical which initiates lipid peroxidation**.** The dose for mice was calculated extrapolating the human therapeutic dose (HTD)

All animals from each group received treatment as per mentioned in experimental study for 7days and Hepatotoxicity induced by using CCl_4 in all mice except normal control group at the end of 7^{th} day. Blood sample has been collected by retro –orbital plexus of all animals and finally all animals were sacrificed. For the histopathological examinations' liver was isolated and examined.

CONCLUSION

Dhatri Avaleha formulation mentioned in Charak-Chikitsasthanawhich is useful in Pandu, Kamala, Halimak is taken for present study. *Dhatri Avaleha* was proved to have hepatoprotective activity against CCl4 induced hepatotoxicity in albino mice. But it was not effective than silymarin.

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