Research Article

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COMPARISON OF HEPATOPROTECTIVE ACTIVITY OF NISHA LAUHA (NL) AND NISHA LAUHA WITHOUT LAUHA BHASMA (NLWL) AGAINST CCL4 INDUCED HEPATOTOXICITY IN WISTAR RATS

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ABSTRACT

Introduction: Many herbal drugs are used to treat liver diseases, but the dose of the herbal drug is high, and they have lesser palatability. An ideal medicine is a medicine that is effective, easy palatable and produces quick action in a low dose. It is possible by adding metals like Lauha *(Iron)* to the herbal drugs. Objective: To compare the hepatoprotective effect of Nisha Lauha (NL) and Nisha Lauha without Lauha Bhasma (NLWL) in experimental rats. Materials and methods: 40 rats were taken divided into five groups, and each group contained eight rats. Among these groups, four groups receive 0.2 ml of injection containing the 0.1 ml CCL_4 plus 0.1 ml liquid paraffin given intraperitoneally for 28 days to induce Hepatotoxicity. Both Test groups received NL and NLWL at a dose of 45mg/kg bd. wt. and 450mg/kg bd. wt. respectively for 28 days. The standard group receives silymarin at a 100 mg/kg bd dose. wt. for 28 days by oral route. The hepatoprotective effect was analyzed using biochemical parameters and histopathological study of the liver. Results: Both the Test and standard groups do not show toxic effects against CCL4 induced hepatotoxicity and lower the dose of the herbal drug due to the addition of Lauha. Conclusion: The result suggests that both test group NL and NL without Lauha Bhasma shows the hepatoprotective activity as equivalent to standard drug silymarin. The addition of Lauha Bhasma to herbal drugs decreases the dose without affecting the drug's efficacy against the hepatoprotective effect.

Keywords: Hepatoprotective, Nisha Lauha, CCL_4

INTRODUCTION

The liver is responsible for multiple metabolic functions and physiological processes such as bile production, energy generation, vitamin storage, and the metabolism of carbohydrates, proteins, and lipids.[1] According to the latest WHO data published in 2017, Liver Disease Deaths in India reached 259,749 or 2.95% of total deaths. The age-adjusted Death Rate is 22.93 per 100,000 population and ranks India #63 globally.[2] The prevalence rate of liver disease in India is 5%.[3] Liver diseases have become a severe health problem because of the broader use of prescribed medication with adverse reactions in today's modern life or drug misuse.[4] Ancient text of Ayurveda described many herbal drugs used in the treatment of hepatic disorder such as Triphala[5] (combination of Amalaki (*Indian gooseberry*), Haritaki (*Terminalia chebula*) and Bibhitaki (*Terminalia bellirica*), Haridra[6] (*Curcuma Longa* Linn.) Daruharidra[7] (*Berberis aristata*), Kutaki[8] (*Picrorhiza kurroa*) have proven hepatoprotective against hepatic disorders. But the dose of herbal formulation is 5- 10 g/day.[9] Hence they have to be used in large quantities.

Metal and minerals are described as kamalahar, such as Shilajatu *(Black bitumen)*,[10] Lauha *(Iron),*[11] Tamra (Copper),[12] etc. Many formulations of lauha like Dhatri Lauha,[13] Navayas lauha[14] Darvyadi lauha[15] Nisha Lauha[16] are manufactured by adding Lauha bhasma (Ash of incinerated Iron) to the herbal formulation. It leads to a reduction in dose, increase palatability and produces quick action.[9] The present study was intended to compare the hepatoprotective activity of Nisha Lauha with and Without Lauha bhasma.

Aims and objectives

1. To compare the hepatoprotective activity of Nisha Lauha (NL) and Nisha Lauha without Lauha Bhasma (NLWL) in Wistar Rats.
2. To evaluate the action of Lauha bhasma on the efficacy and dose of Nisha Lauha (NL) and Nisha Lauha without Lauha Bhasma (NLWL) in Wistar Rats.

MATERIALS AND METHODS

Preparation of Nisha Lauha (NL) and Nisha Lauha without Lauha Bhasma (NLWL)

Raw materials required for Nisha Lauha are purchased from the local Market and standardized as per API standards. Nisha Lauha contains Triphala (A combination of Amalaki (Indian gooseberry), Haritaki (*Terminalia chebula*) and Bibhitaki (*Terminalia bellirica*) in equal quantity), Haridra (*Curcuma Longa* Linn.), Daruharidra (*Berberis aristata*), Kutaki (*Picrorhiza kurroa*) and Lauha Bhasma each in equal amount.[16] Lauha Bhasma was prepared as per the reference of Ayurveda prakasha[17] at Rasashastra and the Bhaishajya kalpana department. All herbal drugs were powdered and mixed with lauha bhasma in mortar and pestle to prepare Nisha Lauha (NL). Nisha Lauha without lauha bhasma (NLWL) was prepared by mixing herbal ingredients in mortar and pestle.

Animal Used

Adult Wistar rats of either sex weighing between 180-200g were used. Ethical approval has been obtained from the Animal Ethics Committee, College of Veterinary & Animal Sciences, Parbhani. (Reference number IAEC/31/18 dt. 11/05/2018.) All animals were housed in a well-ventilated animal house in a propylene cage and 12 hours light-dark cycle (130- 400 Lux), Temperature of 22±3°C with humidity of 30-70 % was maintained. Animal diet was prepared as per the daily nutritional requirement, provided with water ad-libitum.40 Wistar rats of either sex were selected, divided into five groups. After proper labelling for identity, each group with eight animals was kept in separate cages.

Hepatoprotective Activity

The animals were arranged into five groups of eight animals each. Group, I served as normal control receiving the only feed. Group II served as the Negative control group; in this group, Hepatotoxicity is induced by using the CCL_4 (CCL4 0.1 ml+ liquid paraffin 0.1 ml) intraperitoneal for 28 days. In Group III, Hepatotoxicity is induced using the CCL_4 (CCL4 0.1 ml+ liquid paraffin 0.1 ml) intraperitoneal for 28 days and treated with standard drug silymarin at a dose of 100mg/kg bd. wt. orally for 28 days. In both Group IV and V, Hepatotoxicity is induced by using the CCL_4 (CCL4 0.1 ml+ liquid paraffin 0.1 ml) intraperitoneal for 28 days and both test groups Treated with Nisha Lauha (NL) 45mg/kg bd. wt. orally for 28 days and Nisha Lauha without Lauha Bhasma (NLWL) at a dose of 450mg/kg bd. wt. orally for 28 days.

Biochemical Estimation

Blood samples were collected from all groups from the retroorbital plexus. Clear serum samples were carefully drawn and transferred to dry; clean, sterilized serum-containing tubes were stored at -18°C in a deep freeze and used for biochemical estimations.[18] Following biochemical parameters such as Serum alanine transaminase (ALT)(IU/L), serum aspartate transaminase (AST)(IU/L), Serum Alkaline Phosphatase (ALP)(IU/L), and Serum bilirubin (mg/ml) estimated by reported method done on day 0,14th, and 28th.Morphological parameter like changes in the weight of the liver is determined.

Histopathological study

At the end of an experimental trial, tissue samples of liver and kidney were collected for the histopathological study in 10% neutral buffer formalin and processed by routine paraffin embedding technique, were stained with H and E stain. Ethical standards were followed by CPSEA (Committee for Control and Supervision of Experiments on Animals) design described by vogel[19].

Statistical Analysis

Results of the study were expressed as Mean±SE. The data obtained from various parameters from all groups were analyzed by the method suggested by Panse and Sukhatme (1967) using Factorial Randomizes Block Design (FRBD) and completely randomized block design (CRD) as per requirement and interpreted.[20]

RESULT

Table 1: Effect of Nisha Lauha and Nisha Lauha without Lauha bhasma on Biochemical parameters in CCL_4 induced Hepatotoxicity

Parameters	Total Bilirubin (mg/dl)			ALT (IU/L)			AST (IU/L)			ALP (IU/L)		
Group / Days	'0' days	14th day	28th day	'0' days	14th day	28th day	'0' days	14th day	28th day	'0' days	14th day	28th day
Group I Normal	0.65± 0.09	0.63± 0.09	0.69± 0.08	28.71± 3.17	27.05± 1.41	28.68± 1.18	43.22± 3.67	39.74± 1.66	37.88± 1.87	31.18± 4.76	32.19± 3.46	36.79± 1.72
Group II Control (CCL_4 Induced)	0.67± 0.14	3.45± 0.97ab	3.18± 1.12ab	28.48± 1.74	63.27± 1.75b	82.64± 2.55ab	39.67± 2.61	89.31± 1.89b	100.71± 3.22ab	37.13± 3.17	51.62± 1.09	55.46± 1.55
Group III-Standard Group	0.52± 0.17	1.18± 0.13a	1.00 0.05a	24.68± 4.21	43.16± 1.59	37.31± 0.29ab	34.34± 3.05	54.18± 1.83	47.62± 0.60	36.56± 3.41	47.10± 0.35	45.69± 0.22
Group IV-Test Drug- NL	0.64± 0.08	1.25± 0.18a	1.11± 0.13a	25.70± 2.87	43.58± 1.61	38.85± 1.20a	39.46± 3.37	56.22± 2.16	49.32± 1.42	36.06± 3.49	47.57± 0.60	46.04± 0.42
Group V-Test Drug- NLWL	0.55± 0.19	1.19 ± 0.21a	1.00 ± 0.16a	26.97± 1.87	44.64± 1.57	40.97± 1.40a	38.52± 1.11	53.72± 1.37	50.82± 1.65a	37.71± 1.89	47.51± 0.51	46.15± 0.38

NL- Nisha Lauha, NLWL-Nisha Lauha without Lauha Bhasma, CCL_4-Carbontetrachloride, a= level of significance within the group; b= level of significance between groups, Values mean± Standard Error

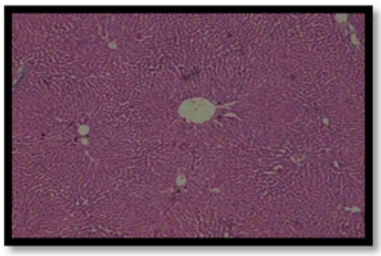
Table 2: Weight of Liver in gram (Mean ± SE) in experimental rats on termination

Treatment	Liver
Group – I Normal	9.52 ± 0.11
Group –II Control (CCL_4 induced)	10.38 ± 0.14
Group – III Standard Control Silymarin	10.28 ± 0.20
Group – IV Test Drug NL	9.84 ± 0.21
Group –V Test Drug NLWL	10.00 ± 0.19

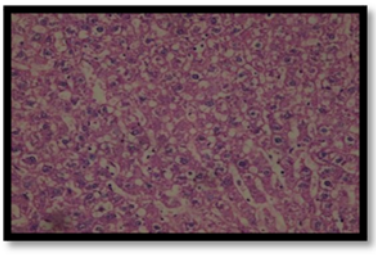
NL- Nisha Lauha, NLWL-Nisha Lauha without Lauha Bhasma, CCL4-Carbontetrachloride, Values mean± Standard Error, N=8 Number of Animals

CCL_4 is a well-known hepatotoxic industrial solvent.[21] CCL_4 commonly used for free radical-induced liver injury. CCL_4 toxicity results from bioactivation of CCL_4 into trichloromethyl free radical by cytochrome P-450 system in liver microsomes and consequently causes lipid peroxidation of membranes of liver without covalent binding, antioxidants and a radical scavenger which affects the cellular permeability of hepatocytes leading to elevated levels of biochemical parameters. The administration of CCL_4 resulted in marked alteration in serum hepatic enzymes such as AST, ALT, ALP and Sr. Bilirubin, which are indicative of hepatic injury.

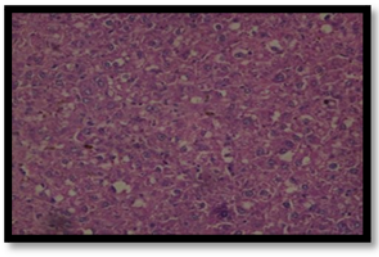
In the present study, a statistically significant increase was observed in the mean of Sr. Bilirubin, ALP, and ALT on day 14th and 28th day in group II, indicative of Hepatotoxicity. Group III, IV and V show no significant increase in ALT, ALP and AST values on the 14th day. At the same time, a decline is observed in the importance of ALT, ALP and AST on the 28th day, which shows the positive effect of the drug.



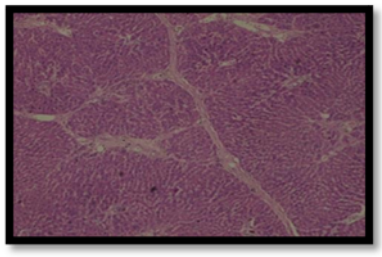
a. Liver of control Group with No hepatic changes



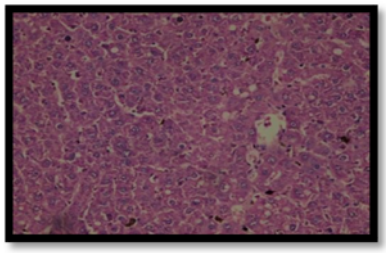
b. Liver with multifocal fatty change and severe degenerative changes in hepatic parenchyma in the rat of group II



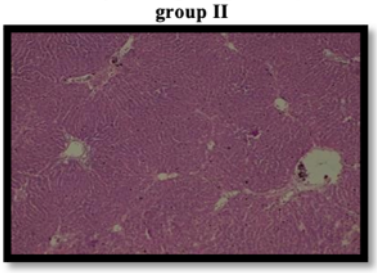
c. liver with mild focal fatty change in hepatic parenchyma in the rat of group II



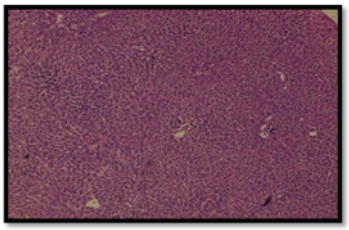
d. Liver with Fibrous tissue proliferation and moderate degenerative changes of hepatic parenchyma in the rat of group II



e. Minimal focal fatty change in the liver of rat of group III



f. Minimal degenerative changes in hepatic parenchyma in the rat of group IV



g. Section of the liver with minimal mononuclear cell infiltration and degenerative changes in hepatic parenchyma in a rat of group V

Fig 1: a. Section of the liver control group; b:c: d: section Of CCL_4 treated group liver; e: CCL_4+ Silymarin treated group; f: CCL_4 + Nisha Lauha; g: CCL_4 + Nisha Lauha without Lauha Bhasma

DISCUSSION

In group III, the IV and V values of Sr. bilirubin on the 14th day statistically increase and declines in Sr. bilirubin's value on the 28th day. The mean value of bilirubin in groups III, IV and V were significantly decreased compared to the 28th-day values of group II. (Table 1)

ALT values in groups IV, V on the 28th day were slightly higher than group III on the 28th day. But the deals decreased compared to values on the 14th day, which indicates the drug's efficacy was compared with standard drug silymarin. (Table 1)

ALP values on days 14th and 28th of group II, IV and V against respective day "0" values were increased, but all the values were observed to be within normal physiological limits. ALP values on day 28th were honoured to be reduced against day 14th values in group III, IV and V as against group I and II animals. Reduction in mean AST value in groups I, III, IV and V were observed as against its day 14th value, indicating the repair in liver function in test drug-treated group animals. (Table 1)

Finally, the animals were sacrificed on the 28th day, and livers were isolated. The weight of each liver was taken, and then histopathology of the liver samples was carried out. No statistically significant alterations in mean absolute liver organ weight were observed in control and treatment group animals. (Table 2)

The histopathological changes in the liver in group II were moderate to severe focal to diffused fatty changes, degenerative changes in hepatic parenchyma, dilatation of central vein,

sinusoidal spaces, and focal necrotic changes, mononuclear cell infiltration and fibrous tissue proliferation in interstitial hepatic lobule. However, the liver sections from groups III, IV and V show minimal fatty change, degenerative changes and congestion in a few sections. (Fig. 1)

In the present study, Test drugs NL and NLWL produce significant hepatoprotective activity equivalent to standard drug Silymarin. The addition of Lauha bhasma to herbal ingredients of Nisha Lauha does not increase the hepatoprotective effect, but it lowers the dose of only herbal drug formulation.

Triphala is one of Nisha Lauha, contains *Emblica officinalis Gaertn., Terminalia belerica* Roxb*. and Terminalia chebula* Retz. They have proven hepatoprotective effects.[22] High content of phenolic compounds and flavonoids in *Terminalia chebula* and *Terminalia belerica* might be responsible for the hepatoprotective activity of Triphala.[23] Inhibition of LPO (Lipid peroxidation) suggested that triphala may exert a stabilizing action on liver cell membranes. Triphala has reduced the increased value of liver enzymes very effectively and have a membrane-stabilizing effect.[24] Amalaki (*Emblica officinalis* Gaertn) appears mediated by its free radical scavenging, antioxidant, anti-inflammatory and modulation of the xenobiotic detoxification process and lipid metabolism.[25] Kutaki (*Picrorhiza kurroa*) maintains an average oxidation-reduction balance and exhibits anti cholestatic activity against various liver-toxic substances.[26] Daruharidra (*Berberis aristata*) has cholagogue, hepato-stimulant that reduces the excretion of excessive formation of bile pigments. It reduces the level of serum enzymes in the blood and decreases the inflammation of the liver.[27] Curcumin is the most common antioxidant constituent of Haridra (*Curcuma longa* Linn.) are used to enhance apoptosis of damaged hepatocytes, which might have protected against the inflammatory effects and fibrogenesis of the liver.[28] Above all, herbal drugs are known for the hepatoprotective effect, but there has been no work on the hepatoprotective effect by adding lauha bhasma. (Ash of incinerated Iron) Here we can say that adding metal and minerals can minimize the dose of herbal drugs without changing the drug's efficacy and with better palatability.

CONCLUSION

The present study concluded that Nisha Lauha (NL) and Nisha Lauha without Lauha Bhasma (NLWL) both drugs have hepatoprotective activity equivalent to silymarin. NL and NLWL show significant hepatoprotective activity at their respective dose. The addition of Lauha bhasma to herbal ingredients (Triphala, Haridra, Daruharidra, Kutaki) reduces the dose of the herbal combination without changing the efficacy of both drugs.

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