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Pharmacological and Toxicological Studies of an Ayurvedic Formulation ("Lauhasava") on the Biological System of Rats and Mice

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Abstract: In this study, the pharmacological and toxicological effects along with possible side effects of the classical ayurvedic formulation "Lauhasava" (LSV), which is used in anemia, were evaluated. During this study, various experiments on body growth rate, organ-body weight ratio, tissue hydration indices and body fat ratio were performed to evaluate its efficacy and toxicity. In the body weight study, the LSV treated rats, irrespective of sexes, showed significantly lower body weights than the control group rats. LSV treated mice of both sexes gained less weight than their control counterparts. The study involving comparison of the relative weights of the major organs of rats and mice revealed some significant results. The percentage of lung to the body weight is significantly increased in both sexes of rats. Liver weight in LSV treated rats of both sexes were observed to be increased. The percentage of kidney weight was increased in both sexes of rats, the result being significant in the case of female rats. The thymus weight was found significantly decreased in both sexes of rats. Significant increase in the weight of rats' ovaries was observed. In the tissue hydration index experiment, only the increase in the female kidney was significant. No significant result was found in the fat content of the whole mice and eviscerated mice.

Key words: LSV, ayurvedic, anti-anemic, pharmacology, toxicology

INTRODUCTION

"Lauhasava" (LSV), popular Ayurvedic OTC drug, is the preparation of "Lauha Bhasma" (roasted iron) along with 11 medicinal plants. Honey and molasses are also used in this preparation. From the ancient time, iron is used in various ways for medication. In anemia, the level of hemoglobin, conjugated molecule of heme and globulin, is lowered than normal level. Iron is the important component of heme. For the treatment of anemia, very common in women of reproductive age, LSV is widely used.

All the medicinal plants used in this formulation are reported individually or jointly for their therapeutic uses. The equi- proportion mixture of *E. officinalis*, *T. chebula* and *T. beleracia* have shown free radical scavenging and radio protective activity (Jagetia *et al*., 2004). According to Ghani (2003), *P. zeylanica* is used in dyspepsia, diarrhea, rheumatoism and parsitic skin diseases. It is also sudorific and antipyretic. *W. fruticosa* is used as astringent (Evans, 2002). Ghani (2003) also reported that *P. nigrum* is pungent, carminative, stimulant, anti-periodic and used in cold, asthma, skin disease, cholera and dyspepsia and *C. rotundus* is anti-inflammatory, anti-pyretic and analgesic. *P. longum* is analgesic, carminative and sedative and used in cough, bronchitis (Ali, 1998). As aromatic stimulant and carminative *Z. officinale* is popular and used in dyspepsia, flatulance colic, vomiting spasm, cold, cough, asthma etc. (Ali, 1998). The hypoglycemic and anti-fertility activities of *E. ribes* are mentioned by Ghani (2003) and anti-spasmodic activity of *T. ammi* is mentioned by Ali (1998). The pharmacological activity and therapeutic uses of individual ingredients are well established but no report was found on the formulation of LSV. This study was performed in an effort to evaluate the safety of this drug according to modern toxicological parameters so as to fully fathom the safety of this Ayurvedic drug as a hematinic for Alternative Medical Care.

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MATERIALS AND METHODS

LSV, included in the Bangladesh National Formulary of Ayurvedic Medicine 1992 (page-128), was collected from Sri Kundeswari Aushadhalaya Ltd., Chittagong. For all the pharmacological studies, the drug was administered via per oral route at a dose of 40 mL kg^{-1}. Swiss-Webster strain male and female mice (20-40 g body weight) were used for the pharmacological experiments (Table 1). For some experiments, Sprague-Dawley albino rats were also employed. According to Glasnapp and Poggio (1985) un-paired t-tests were done for statistical significance tests. $p = 0.05$ was taken to be the level of significance.

Body weight study: LSV was administered (40 mL kg^{-1}) once daily into the rat and mice for a period of 55 and 51 days respectively in order to assess the effects of the formulation on the body weight growth rates of rats and mice of both sexes. An equal number of animals of the same species were also maintained as the control group, which was given only distilled water. Body weight was recorded at regular intervals (2-3 days) until the treatment period was complete. Both groups of animals were kept under similar environmental conditions and provided with enough food and water throughout the experiment. In statistical analysis, the growth rate, expressed as percent increment in the body weight of the treatment group, was compared with that of the control group (Weinstock *et al*., 1997).

Tissue hydration index: Excised portion of heart, lung, liver and spleen (portion not subjected to histological evaluation); one entire kidney and one testis (in case of male) were weighed in an electronic balance (Shimadzu) after gently blotting dry, thus giving the wet weight of the tissue (W) (Weinstock *et al*., 1997). These samples were dried at 110°C for 60 h and then weighed to determine the dry tissue weight (D). Tissue hydration index (HI) was calculated as:

$$HI = (W-D)\times 100/W$$

Estimation of fat, water and lean mass: The determination of the fat content in the whole animals as well as in the eviscerated (devoid of the internal visceral organs) carcasses was performed by carrying out post-mortem analysis of the experimental animals following chronic oral administration with daily 40 mL kg^{-1} doses of the drug to Swiss-Webster mice of both sexes. An equal number of control animals of both sexes were maintained. The control group animals were administered with equivalent amount of distilled water. At the end of 51 days treatment period, the mice of both the LSV and control groups were sacrificed. A total of 24 animals including male and female (drug treated and control) were dissected by removing all the internal organs to end up into eviscerated carcasses. The remaining 24 animals were taken as the group for whole body analysis of fat. After sacrifice, this group of mice was not dissected; instead, a simple abdominal incision was made on each of them. Thereafter, the whole mice as well as the eviscerated mice carcasses were oven dried at 110°C for a period of 3 days. The weight difference taken before and after drying gives the water content of the body. Carcasses were then scissored into small fragments and the lipids extracted with Folch's solvent (Chloroform/Methanol in the ratio of 2:1, v/v). Lipid of whole mice carcasses were extracted thrice with chloroform/methanol mixture and extraction with chloroform alone was performed twice before the completion of the process. Eviscerated carcasses were extracted twice with Folch's solvent and then twice again with just chloroform. The lipid containing aliquots were

Table 1: The plants and ingredients used in the formulation of Lauhasava (LSV)

Name of plants/ingredients	Used parts	Botanical name	Family	Amount used
Loha cuma (Rosted iron)				192 g
Guda (Molasses or brawn sugar)				4.800 kg
Kasaudra/Madhu (1 honey)				3.072 kg
Water				24.576 L
Amalaki	Fruit powder	*Embelica officinalis*	Euphorbiaceae	192 g
Bibhitaka	Fruit powder	*Terminalia beleracia*	Combretaceae	192 g
Citraka	Root	*Plumbago zeylanica*	Plumbaginaceae	192 g
Dhataki kusuma	Flower	*Woodfordia fruticosa*	Lythraceae	192 g
Haritaki	Fruit powder	*Terminalia chebula*	Combretaceae	192 g
Marica	Fruit powder	*Pipper nigrum*	Piperaceae	194 g
Mustaka	Rhizome	*Cyperus rotundus*	Cyperaceae	192 g
Pippali	Fruit	*Pipper longum*	Piperaceae	192 g
Sunthi	Rhizome	*Zingiber officinale*	Zingiberaceae	192 g
Vidanga	Fruit powder	*Embelica ribes*	Myrsinaceae	192 g
Yavanika	Fruit powder	*Trachyspermum ammi*	Apiaceae	192 g

collected in numbered and readily identifiable beakers. The lipid aliquots as well as the extracted mice homogenate were dried for 24 h on water bath, followed by oven-drying at 110°C for 2 days and then weighed in an electronic balance. The weight of the dried aliquots represents the fat content of the animal in grams, whereas the weight of the dried homogenate is a measure of the lean body mass (Chohen *et al*., 2002; Laugero and Moberg, 2000).

RESULTS AND DISCUSSION

Body growth rate, expressed in percentage, was studied after chronic administration of LSV at a dose of 40 mL kg^{-1}. In the Over all growth analysis of male rats, the body weights of the LSV treated rats were found to be lower than those of the control group rats. The decrease in the body weight on the 10th ($p = 0.047$) and 55th ($p = 0.011$) days were found to be statistically significant. From the 7th day onwards, the growth rates of the LSV treated female rats were observed to decline and the growth rates continued to be lower than the control group till the final day of observation. However, the result was statistically highly significant only on the 55th day ($p = 0.004$) (Table 2).

In the initial two weeks, LSV treated male mice gained slightly more weight than their control counterparts (statistically not significant), but from then till the end of 51 days study period, there was a consistent decrease in the body growth rate though it was statistically insignificant. The decline in the growth rates of the LSV treated female mice was much more prominent, albeit statistically insignificant, than the LSV treated male mice. The decrease in body weight gain for LSV treated female mice was very prominent in between the 3rd and the 7th weeks (Table 3).

In organ/body weight ratio determination, result shows negligible decrease in heart weight ratio of LSV treated male rats and negligible increase in heart weight ratio of LSV treated female rats. The percentage of lung weight to the body weight is increased in both male and female rats in a significant manner (p-values 0.025 and 0.019, respectively). LSV treated rats, both male and female, showed an increase in the liver weight. The increase in the female rats was prominent and was very highly significant with a p-value of 0.001. Liver weight in the mice of LSV treated group, irrespective of sex, was found to be insignificantly lower than those of control group mice. Like liver, percentage of kidney weight to the body weight was increased in both sexes of rats where the

Table 2: The effect of LSV on the body weight growth study of rats (Dose 40 mL kg^{-1})

Study period	Sex	Group	Mean±SEM	t/p
55 days	M	CTR (n = 9)	188.045±11.614	2.886/0.011*
		LSV (n = 9)	138.984±12.421	
	F	CTR (n = 10)	128.828±10.596	3.310/0.004**
		LSV (n = 9)	84.378±7.772	
10 days	M	CTR (n = 9)	23.810±6.229	2.151/0.047*
		LSV (n = 9)	8.674±3.274	
	F	CTR (n = 10)	15.585±5.566	1.706/0.105
		LSV (n = 9)	5.544±1.913	
7 days	M	CTR (n = 9)	6.357±1.605	1.812/0.089
		LSV (n = 9)	1.320±2.270	
	F	CTR (n = 10)	2.218±1.960	0.470/0.644
		LSV (n = 9)	1.082±1.413	

*$p \leq 0.05$, **$p \leq 0.01$

Table 3: The effect of LSV on the over all body weight growth study of mice (Dose 40 mL kg^{-1})

Study period	Sex	Group	Mean±SEM	t/p
51 days	M	CTR (n = 12)	107.676±5.982	-0.219/0.828
		LSV (n = 11)	96.315±6.542	
	F	CTR (n = 11)	70.551±4.807	0.711/0.485
		LSV (n = 12)	41.988±20.011	
7 days	M	CTR (n = 12)	30.938±1.257	0.225/0.824
		LSV (n = 11)	30.233±2.974	
	F	CTR (n = 11)	23.710±2.855	0.712/0.484
		LSV (n = 12)	15.502±10.700	
4 days	M	CTR (n = 12)	20.053±1.843	1.281/0.215
		LSV (n = 11)	20.723±2.478	
	F	CTR (n = 11)	15.412±2.660	1.332/0.197
		LSV (n = 12)	7.803±9.925	

result of female LSV rats is significant ($p = 0.035$). Unlike rats, the relative kidney weights of the LSV-treated mice of both the sexes were found to decrease. A slight increase was observed in spleen weight of LSV treated male rats whereas no change was observed in female rats; both these results being statistically insignificant. Significant decrease in the thymus weight was found in both the male ($p = 0.011$) and the female rats ($p = 0.005$). There was negligible change in the testis weight ratio of the LSV treated rats and mice. Ovaries of rats were profoundly increased in weight gaining significant result ($p = 0.039$) (Table 4, 5).

In the tissue hydration index determination, the water content of the LSV treated livers of the rats of both sexes were noted to be the same as their control counterparts. Water content of the livers of drug-treated mice of both sexes showed an insignificant increase. A statistically insignificant increase in the water content of the spleen of male rats and decrease in the female rats were observed. The results were reversed in case of mice. Kidney hydration index was found to be increased in the LSV treated rats of both the sexes, where the result of female rats was significant ($p = 0.05$). On the other hand, a slight decrease in the kidney hydration indices of male mice and slight increase in those of female mice were observed. LSV caused a slight decrease in the water content of the heart

Table 4: The effect of LSV on the tissue hydration index and histopathological analysis of rats (Dose 40 mL kg^{-1})

			Organ body weight ratio		Organ water content	
Parameters	Sex	Groups	Mean±SEM	t/p	Mean±SEM	t/p
Heart	M	CTR (n = 9)	0.286±0.007	0.754/0.463	80.361±1.056	0.83/0.42
		LSV (n = 8)	0.279±0.004		79.178±0.944	
	F	CTR (n = 10)	0.270±0.01	-1.330/0.201	76.653±0.186	1.03/0.31
		LSV (n = 9)	0.286±0.009		71.546±5.209	
Lung	M	CTR (n = 9)	0.505±0.008	-2.490/0.025*	78.420±0.465	0.04/0.96
		LSV (n = 8)	0.570±0.026		78.400±0.163	
	F	CTR (n = 10)	0.530±0.01	-2.500/0.019*	76.882±0.452	0.73/0.47
		LSV (n = 9)	0.595±0.02		71.518±7.699	
Liver	M	CTR (n = 9)	3.040±0.063	-0.292/0.775	71.332±0.498	-0.36/0.72
		LSV (n = 8)	3.150±0.393		71.530±0.123	
	F	CTR (n = 10)	2.762±0.08	-4.286/0.001***	70.840±0.359	
		LSV (n = 9)	3.147±0.04		71.042±1.271	
Kidney	M	CTR (n = 9)	0.302±0.005	-1.997/0.064	73.339±2.534	-1.08/0.29
		LSV (n = 8)	0.315±0.007		76.287±0.271	
	F	CTR (n = 10)	0.294±0.008	-2.293/-0.035	76.100±0.292	-2.03/0.05*
		LSV (n = 9)	0.320±0.007		84.852±4.527	
Spleen	M	CTR (n = 9)	0.234±0.007	-1.175/0.258	65.774±9.808	-0.98/0.34
		LSV (n = 8)	0.299±0.059		74.458±2.282	
	F	CTR (n = 10)	0.240±0.01	-0.003/0.998	77.809±3.742	1.34/0.19
		LSV (n = 9)	0.240±0.009		58.821±14.363	
Thymus	M	CTR (n = 9)	0.202±0.010	2.893/0.011*		
		LSV (n = 8)	0.160±0.010			
	F	CTR (n = 10)	0.228±0.010	3.245/0.005**		
		LSV (n = 9)	0.181±0.007			
Testis/Ovaries	M	CTR (n = 9)	0.5945±0.021	-0.478/0.639		
		LSV (n = 8)	0.6293±0.074			
	F	CTR (n = 10)	0.037±0.003	-2.240/0.039*		
		LSV (n = 9)	0.046±0.003			
		LSV (n = 5)				

*p≤0.05, **p≤0.01, ***p≤0.001

Table 5: The effect of LSV on the tissue hydration index and histopathological analysis of mice (Dose 40 mL kg^{-1})

			Organ body weight ratio		Organ water content	
Parameters	Sex	Group	Mean±SEM	t/p	Mean±SEM	t/p
		LSV (n = 9)	0.046±0.003			
Liver	M	CTR (n = 6)	5.098±0.188	0.794/0.447	71.607±0.244	-1.19/0.26
		LSV (n = 5)	4.830±0.294		72.006±0.217	
	F	CTR (n = 6)	4.319±0.096	1.054/0.319	71.732±0.493	-1.56/0.15
		LSV (n = 5)	3.953±0.367		75.591±3.025	
Kidney	M	CTR (n = 6)	0.5927±0.016	1.877/0.093	78.275±0.693	0.82/0.43
		LSV (n = 5)	0.544±0.020		77.628±0.183	
	F	CTR (n = 6)	0.4449±0.017	0.250/0.808	79.290±1.053	-1.15/0.28
		LSV (n = 5)	0.4379±0.023		81.843±2.252	
Spleen	M	CTR (n = 6)	0.405±0.05	0.445/0.667	78.275±0.244	1.60/0.14
		LSV (n = 5)	0.379±0.018		77.696±0.264	
	F	CTR (n = 6)	0.3997±0.037	-0.473/0.648	78.282±1.014	-1.15/0.28
		LSV (n = 5)	0.4522±0.114		81.875±3.578	
Testis	M	CTR (n = 6)	0.2737±0.013	0.325/0.753	85.587±0.284	1.88/0.09
		LSV (n = 5)	0.267±0.016		83.610±0.08547	
	F	CTR (n = 6)				
		LSV (n = 5)				

Table 6: The effect of LSV on the estimation of fat, water and lean mass of mice (Dose 40 mL kg^{-1})

Study animal	Sex	Group	Water (%)	Fat (%)	Non fat (%)
Whole mice	Male mice	CTR (n = 5)	65.890±1.40	9.470±1.68	90.530±1.68
		LSV (n = 5)	57.790±3.58	12.880±1.34	87.120±1.14
		t/p	1.950/0.083	-1.743/0.125	1.743/0.125
	Female mice	CTR (n = 5)	61.700±1.250	14.907±1.715	85.092±1.715
		LSV (n = 5)	60.575±2.379	12.301±1.810	87.698±1.810
		t/p	0.396/0.701	1.045/0.327	-1.045/0.327
Eviscerated mice	Male mice	CTR (n = 5)	60.450±6.95	7.380±1.26	92.610±1.26
		LSV (n = 6)	47.150±12.81	8.910±1.85	91.080±1.85
		t/p	0.960/0.36	-0.700/0.500	0.700/0.50
	Female mice	CTR (n = 6)	57.986±1.802	16.897±1.129	83.102±1.129
		LSV (n = 6)	50.538±10.310	11.421±2.662	71.911±14.447
		t/p	0.683/0.510	1.893/0.088	0.772/0.458

of the rats of both the sexes; however the results were not significant. The lung hydration index was found unchanged in the LSV treated male rats, whereas in the case of female rats, there was a slight decrease. LSV was observed to cause a slight decrease in the testis hydration index of the male mice which was not significant (Table 4, 5).

In the fat content experiment, the percentage of water was slightly decreased for whole mice of both sexes. No statistical significant findings were present in case of eviscerated mice either. Slight increase observed in the percentage of fat in male and slight decrease observed in female were insignificant. The same trend was observed in eviscerated mice. There were no significant changes in the percentage of lean mass in the whole mice and eviscerated mice, both male and female (Table 6).

REFERENCES

Ali, M., 1998. Text Book of Pharmacognosy. 1st Edn. CBS Publishers and Distributors, New Delhi, ISBN: 81239 0278 6.

Cohen, P., M. Miyazaki, N.D. Socci, A.H. Greenberg and W. Liedtke *et al*., 2002. Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. Science, 297: 240-243.

Evans, W.C., 2002. Trease and Evans Pharmacognosy. 5th Edn., W.B. Saunders, ISBN: 07020 2617 4.

Ghani, A., 2003. Medicinal Plants of Bangladesh with Chemical Constituents and Uses. 2nd Edn., Asiatic Society of Bangladesh, ISBN: 984 512 348 1.

Glasnapp, D.R. and J.P. Poggio, 1985. Essentials of Statistical Analysis for the Behavioral Sciences. 1st Edn., Charles E. Merrill Publishing Company, London, ISBN: 0675203694.

Jagetia, G.C., K.J. Malagi, M.S. Baliga and P.V. Triphala, 2004. An ayurvedic rasayana drug, protects mice against radiation-induced lethality by free-radical scavenging. J. Alternat. Complement. Med., 10: 971-978.

Laugero, K.D. and G.P. Moberg, 2000. Effects of acute behavioral stress and LPS-induced cytokine release on growth and energetics in mice. Physiol. Behav., 68: 415-422.

Weinstock, P.H., S.L. Frank, L.C. Hudgins, J.M. Friedman and J.L. Breslow, 1997. Lipoprotein lipase controls fatty acid entry into adipose tissue, but fat mass is preserved by endogenous synthesis in mice deficient in adipose tissue lipoprotein lipase. Proc. Natl. Acad. Sci. USA, 94: 10261-10266.