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Toxicological Studies of Lingha Chendooram-1; a Siddha Drug

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Lingha chendooram-1, a widely used Siddha drug was evaluated for acute, sub-acute and chronic toxicity in rats with reference to histopathological, haematological, parameters and detection of mercury from the tissues of liver and kidney. The drug showed acute toxicity from 100 mg onwards. But it produced sub-acute and chronic toxic effects from 50 mg onwards and elevation in blood urea level. Feed and water intake failed to reveal any marked changes in sub-acute and chronic toxicity studies. Mercury was detected from 75 mg with concurrent increase in dose in both sub-acute and chronic toxicity studies. The histopathological lesions were non-specific when compared to microscopic changes along with the drug dosages. In the present study the drug possessed no toxic effect below 20 mg.

Lingha chendooram-1 is a widely used drug in Siddha system of medicine. It has been used in therapeutic management for delirious fevers, arthritis and anaemia[1]. It is prepared from cinnabar (lingham), the chief ore of mercury. Cinnabar is identified as mercury (II) sulphide. Generally there are two types of lingha chendooram, lingha chendooram-1 and lingha chendooram-2 based on the preparation methods[1]. Lingha chendooram-1 is prepared by titration process by triturating cinnabar with fresh juice of *Citrullus colocynthis* Schrad and lingha chendooram-2 is prepared by calcination (Pudam) process by adding benzoin and camphor with cinnabar. Pharmacological and toxicological studies of lingha chendooram-2 were reported previously[2,3]. Drug standardisation and pharmacological studies of lingha chendooram-1 has been carried out[1]. Pharmacological studies proved lingha chendooram-1 to be a potent antipyretic, analgesic and antiinflammatory drug, whereas, lingha chendooram-2 possessed hypothermic and mild analgesic and antiinflammatory activities. But there is no work available on the toxicological effects of lingha chendooram-1. The present investigation reports the acute, sub-acute and chronic toxicity studies of this drug in albino rats.

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MATERIALS AND METHODS

Lingha chendooram-1 was prepared from cinnabar following standard methods[1]. Toxicity studies were carried out according to standard procedures[4-6]. For the present study, adult, healthy albino rats of either sex weighing between 150-200 g were used. They were grouped into six animals per group. For the acute toxicity study, the drug was fed orally in doses of 50, 100, 200, 500 and 1000 mg/kg body weight suspended in 2 ml of honey and a pinch of gum tragacanth. For sub-acute and chronic toxicity studies the drug was administered orally in doses of 10, 20, 50, 75 and 100 mg/kg body weight suspended in 2 ml of honey and a pinch of gum tragacanth. For acute, sub-acute and chronic toxicity studies, animals were kept under observation for 72 h, one month and six months, respectively. Throughout the study all animals were maintained under standard conditions and had access to pelleted animal feed and water *ad libitum*. One group of animals was treated as control and was fed with honey and gum tragacanth without drug. For sub-acute and chronic studies, individual body

weight, food and water-intake were noted for all animals. After the course of study, animals were sacrificed and opened for gross observations. Blood was collected for haematological and biochemical studies by carotid bleeding. It was subjected to the following haematological studies[7]: hemoglobin, total white blood cells, differential counts (neutrophils, lymphocytes, eosinophils, basophils and monocytes), blood glucose and blood urea level. The liver and kidney were dissected out for histopathological studies. They were fixed in 10% formalin and paraffin sections were made. Sections were stained by haematoxylin and eosin (HE) for biopsy studies[8]. Fresh tissue samples of liver and kidney were used for estimation of mercury[9,10]. Mercury was extracted from the digest with chloroform solution of dithiazone and the extract was shaken with sulphuric acid to transfer mercury to aqueous form. Mercury was determined from the latter calorimetrically at pH 6 by the mixed colour dithiazone method.

RESULTS AND DISCUSSION

In the acute toxicity study, the animals in the test group did not manifest any signs of toxicity and no deaths were observed up to a dose of 50 mg. One rat died at the dose of 100 mg, 2 at 200 mg, 4 at 500 mg and all rats at 1 g/kg body weight. The therapeutic dose (ED_{50}) for antipyretic, analgesic and antiinflammatory was found to be 20 mg in rats[1]. The lethal dose (LD_{50}) was found to be 255.9±61.8 mg. Therefore, the therapeutic index of lingha chendooram-1 for the antipyretic, analgesic and antiinflammatory activities can be regarded as 12.85. Since this drug has a high therapeutic index, it is considered to be a relatively safe drug. There were no behavioral changes noticed at any dose upto 100 mg/kg. The body weight of Wistar strain of albino rats in acute, sub-acute and chronic toxicity studies was found to be uniformly increased. Statistically significant difference in body weight was not noted in both control and treated groups (Table 1).

Haematological parameters for sub-acute toxicity studies are tabulated in Table 2. Blood glucose, blood urea and percentage of mercury in liver and kidney are tabulated in Table 3. The drug did not cause any death upto a dose of 50 mg but at doses of 75 and 100 mg, mortality was 16.7% and 33.3%, respectively. The body weight was uniformly

TABLE 1: BODY WEIGHT IN ACUTE, SUB-ACUTE AND CHRONIC TOXICITY STUDIES.

	Vehicle		50 mg/kg		100 mg/kg		200 mg/kg		500 mg/kg		1000 mg/kg	
Acute toxicity study	Initial	48 h	Initial	48 h	Initial	48 h	Initial	48 h	Initial	48 h	Initial	48 h
	165.2 ± 4.00	164.8 ± 4.31	171.2 ± 5.28	170.3 ± 5.53	167.3 ± 3.76	166.0 ± 4.27	172.8 ± 5.33	169.0 ± 5.55	158.0 ± 4.48	156.0 ± 4.49	167.5 ± 4.20	165.0 ± 3.96
	Vehicle		10 mg/kg		20 mg/kg		50 mg/kg		75 mg/kg		100 mg/kg	
Sub-acute toxicity study	Initial	After 1 m	Initial	After 1 m	Initial	After 1 m	Initial	After 1 m	Initial	After 1 m	Initial	After 1 m
	165.2 ± 4.44	187.8 ± 5.30	168.0 ± 3.71	191.5 ± 4.24	167.0 ± 3.24	190.3 ± 3.48	165.5 ± 4.42	190.5 ± 4.54	169.4 ± 5.89	191.0 ± 4.58	169.0 ± 1.96	194.0 ± 8.28
	Vehicle		10 mg/kg		20 mg/kg		50 mg/kg		75 mg/kg		100 mg/kg	
Chronic toxicity study	Initial	After 6 m	Initial	After 6 m	Initial	After 6 m	Initial	After 6 m	Initial	After 6 m	Initial	After 6 m
	168.3 ± 4.05	199.8 ± 4.46	168.0 ± 4.04	206.5 ± 4.51	170.8 ± 5.39	206.8 ± 5.39	164.4 ± 3.41	200.8 ± 3.65	163.3 ± 6.01	210.0 ± 7.64	160.0 ± 7.28	207.5 ± 2.50

Each value is expressed as mean ± S.E.M. of 6 observations of lingha chendooram-1 at various dose levels.

increased steadily in both the control and treated animals. There was no marked variation in the food and water intake. Haematological studies and blood glucose were within normal range but blood urea was increased with increase in dosage. Mercury was detectable in both liver and kidney tissues of the animals treated with 75 and 100 mg of the drug, which was statistically significant ($P<0.01$). The presence of mercury in kidney and liver implies that it may lead to renal and hepatic damages as the pathological level of mercury depends upon many factors, namely type of mercury compound, duration of exposure, route of administration, the animal species, their age and sex[11].

The kidney of control and treated animals showed normal histopathological features at 10 and 20 mg doses. Animals treated with 50 mg/kg dose, showed normal glomeruli, extensive necrosis of tubular epithelium and tubules filled with proteinaceous exudate along with interstitial inflammation.

The liver of animals treated with 10 and 20 mg/kg showed non-specific dilatation of sinusoids with infiltration of mononuclear cells. Above 50 mg/kg dose, the liver showed marked dilatation of sinusoids, dilated portal traits, congested blood vessels, infiltration with mononuclear cells and eosinophilic cast and dilated central vein when compared to normal.

Chronic studies with respect to the mortality rate, haematological studies and level of glucose and urea in blood and mercury in liver and kidney are tabulated in Table 4 and 5, respectively. The drug did not cause any death

TABLE 2: HAEMATOLOGICAL PARAMETERS IN SUB-ACUTE TOXICITY STUDIES.

Treatment (mg/kg)	Sample size	Haemo globin (g%)	Total W.B.C. (1000/mm³)	Differential count (%)				
				Neutro-phils	Lymph-ocytes	Eosino-phils	Mono-cytes	Baso-phils
Vehicle	6	16.40 ± 0.35	9.90 ± 0.19	31.50 ± 1.09	64.50 ± 0.76	2.67 ± 0.22	0.83 ± 0.31	0.50 ± 0.22
10	6	15.35 ± 0.30	9.57 ± 0.19	29.83 ± 0.65	67.83 ± 0.70	1.67 ± 0.21	0.33 ± 0.21	0.33 ± 0.21
20	6	15.20 ± 0.29	10.21 ± 0.18	31.50 ± 0.50	66.33 ± 0.67	1.00* ± 0.00	0.67 ± 0.21	0.50 ± 0.34
50	6	14.93* ± 0.22	9.92 ± 0.94	29.50 ± 0.72	67.33* ± 0.36	1.50* ± 0.22	1.67 ± 0.17	0.50 ± 0.22
75	5+	15.08 ± 0.31	9.80 ± 1.00	33.40 ± 0.60	63.60 ± 0.81	1.40* ± 0.25	1.00 ± 0.00	0.60 ± 0.25
100	4+	15.00* ± 0.26	9.96 ± 0.20	35.75 ± 0.48	61.50 ± 0.65	1.25* ± 0.25	1.00 ± 0.00	0.50 ± 0.29
Nornal range		14 - 17.5	9.5 - 11.2	20 - 35	55 - 72	1 - 6	2 - 6	1 - 2

Each value is expressed as mean ± S.E.M. of 6 observations of lingha chendooram-1 and honey as vehicle,+ one animal died at 75 mg/kg and two died at 100 mg/kg doses during the course due to toxicity; *$P< 0.01$ is considered significant as compared to control by Students t-test.

TABLE 3: BLOOD GLUCOSE, UREA ESTIMATION AND MERCURY PERCENTAGE IN LIVER AND KIDNEY IN SUB ACUTE TOXICITY STUDIES.

Treatment (mg/kg)	Sample size	Blood glucose (g%)	Blood urea (g%)	Mercury content in 100 g of tissue	
				Liver	Kidney
Vehicle	6	68.17 ± 2.51	31.33 ± 1.33	0.00 ± 0.00	0.00 ± 0.00
10	6	68.33 ± 1.91	31.00 ± 1.36	0.00 ± 0.00	0.00 ± 0.00
20	6	72.00 ± 1.57	28.33 ± 0.71	0.00 ± 0.00	0.00 ± 0.00
50	6	75.83 ± 1.60	*46.17 ± 0.60	0.00 ± 0.00	0.00 ± 0.00
75	5+	*78.60 ± 1.88	*49.60 ± 0.68	*0.86 ± 0.09	*1.00 ± 0.09
100	4+	*80.00 ± 1.80	*55.00 ± 0.87	*1.44 ± 0.09	*1.70 ± 0.05
Normal range		58-85	20-44	Nil	Nil

Each value is expressed as mean ± S.E.M. of 6 observations of lingha chendooram-1 and honey as vehicle,+ one animal died at 75 mg/kg and two died at 100 mg/kg doses during the course due to toxicity; * $P < 0.01$ is considered significant as compared to control by Students t-test.

upto a dose of 20 mg but at 50, 75 and 100 mg/kg, 16.7%, 50% and 66.7% mortality was observed. The mortality ratio increased with increase in dose. There was a steady increase in the body weight of the treated animals upto 50 mg but it decreased in 75 and 100 mg/kg doses. Feed and water intake did not reveal any change. Blood urea was statistically significant from 50 mg/kg and other haematological parameters observed were within the normal range. Presence of mercury was significant ($P<0.01$) in both liver and kidney above 50 mg/kg. The histopathological studies of kidney of control group animals showed normal features but treated animals above 20 mg dose showed normal glomeruli with focal mesangial proliferation, focal sclerosis of the glomeruli, cloudy swelling of the tubules, interstitial oedema and focal interstitial haemorrhage. The liver of treated animals showed

TABLE 4: HAEMATOLOGICAL PARAMETERS IN CHRONIC TOXICITY STUDIES.

Treatment (mg/kg)	Sample size	Haemo-globin (g%)	Total W.B.C. (1000/mm³)	Differential count (%)				
				Neutro-phils	Lymph-ocytes	Eosino-phils	Mono-cytes	Baso-phils
Vehicle	6	15.08 ± 0.33	9.86 ± 0.99	30.67 ± 0.99	64.50 ± 1.02	2.33 ± 0.33	1.67 ± 0.21	0.83 ± 0.21
10	6	15.67 ± 0.36	9.80 ± 0.99	30.83 ± 0.91	64.17 ± 1.01	2.33 ± 0.33	1.67 ± 0.21	1.00 ± 0.26
20	6	16.16 ± 0.33	9.87 ± 0.14	27.67 ± 2.54	64.67 ± 2.87	3.00 ± 0.37	4.00* ± 0.45	0.67 ± 0.21
50	5+	15.90 ± 0.29	10.00 ± 0.79	28.80 ± 1.39	66.80 ± 2.08	2.20 ± 0.20	1.80* ± 0.58	0.40 ± 0.24

75	3+	15.33 ± 0.44	9.97 ± 0.91	29.33 ± 0.88	67.67 ± 0.67	1.33 ± 0.33	1.00* ± 0.00	0.67 ± 0.33
100	4+	15.50 ± 0.50	9.90 ± 0.31	31.00 ± 3.00	66.00 ± 3.00	1.50 ± 0.50	1.00 ± 0.00	0.50 ± 0.50
Normal range		14 - 17.5	9.5 - 11.2	20 - 35	55 - 72	1 - 6	2 - 6	1 - 2

Each value is expressed as mean ± S.E.M. of 6 observations of lingha chendooram-1 and honey as vehicle, +one animal died at 50 mg/kg, three died at 75 mg/kg and four died at 100 mg/kg doses during the course due to toxicity; * P< 0.01 is considered significant as compared to control by Students t-test.

ballooning with feathery degeneration of the hepatocytes, focal steatosis, congestion of central vein, congested sinusoids, prominent kupffer cells and scanty inflammatory portal tract when compared to normal. Mercury was detectable in both liver and kidney tissues from animals treated with a dose of 50 mg onwards and concurrent increase as the dose was increased.

The histopathological lesions were non-specific when compared to the microscopic changes along with drug doses. The histopathological studies showed toxic effects on renal parenchyma vessels, necrosis and also inflammatory changes in the liver cells. Haematological findings also confirmed the elevation in blood urea level. So, the drug is hepatotoxic and nephrotoxic and not suitable for long-term therapy. However, the drug possesses no toxic effect below 20 mg/kg body weight. Similar work on the neuronal response with crude drug cinnabar has also been reported[11].

But lingha chendooram-2 did not show any acute toxic effects such as behavioral changes, mortality upto 500 mg/kg, but produced sub-acute toxicity from the dose of 200 mg/kg onwards[3]. There was not much difference in

TABLE 5: BLOOD GLUCOSE, UREA ESTIMATION AND MERCURY PERCENTAGE IN LIVER AND KIDNEY IN CHRONIC TOXICITY STUDIES.

Treatment (mg/kg)	Sample size	Blood glucose (g%)	Blood urea (g%)	Mercury content in 100 g of tissue	
				Liver	Kidney
Vehicle	6	58.67 ± 2.11	26.50 ± 0.88	0.00 ± 0.00	0.00 ± 0.00
10	6	62.83 ± 1.68	27.50 ± 1.18	0.00 ± 0.00	0.00 ± 0.00
20	6	61.33 ± 1.33	*31.00 ± 0.86	0.00 ± 0.00	0.00 ± 0.00
50	5+	58.20 ± 1.28	*42.20 ± 1.20	*0.42 ± 0.04	*0.68 ± 0.04
75	3+	61.67 ± 2.03	*49.67 ± 2.19	*0.54 ± 0.03	*0.78 ± 0.06
100	2+	58.50 ± 1.50	*60.00 ± 4.96	*1.08 ± 0.15	*1.23 ± 0.14
Normal range		58-85	20-44	Nil	Nil

Each value is expressed as mean ± S.E.M. of 6 observations of lingha chendooram-1 and honey as vehicle, +one animal died at 50 mg/kg, three died at 75 mg/kg and four died at 100 mg/kg doses during the course due to toxicity; * P< 0.01 is considered significant as compared to control by Students t-test

haematological parameters carried out on the two drugs. Biochemical parameters were not reported for lingha chendooram-2. In lingha chendooram-1 treatment, mercury was detectable above 50 mg/kg dose level in liver and kidneys of treated animals. However, at the therapeutic dose (20 mg/kg) there is no trace of mercury in liver and kidney. Histopathological changes were non-specific because the changes observed in the tissues did not show remarkable and irreversible changes in the tissues and the microscopic changes are not proportionate to the dose administered. For lingha chendooram-2, further studies have to be conducted to evaluate dose dependency. However, lingha chendooram-1 at a dose level of 20 mg/kg body weight is considered to be safe and assessed parameters are within normal limits and the drug has a high therapeutic index. It is concluded that long term clinical trials are also necessary to sustain the efficacy of the drug.

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