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PRE-CLINICAL TOXICITY STUDIES OF *HOLARRHENA ANTIDYSENTRICA* STEM BARK IN MICE AND RATS

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ABSTRACT

The present study has been conducted to assess the Pre-Clinical safety of *Holarrhena antidysentrica* stem bark in mice and rat model. Albino mice (Swiss) were used in graded doses of *Holarrhena antidysentrica* by oral routes. Acute toxicity of *Holarrhena antidysentrica* showed normal behaviour and no mortality up to the 14th day. The animal treated with 2000, 1000 & 500 mg/kg, p.o. showed dull, writhing and 30% mortality recorded within 96 h. Effect of *Holarrhena antidysentrica* on haematological, biochemical and histopathological parameters in sub –acute toxicity was carried out. Sub-acute toxicity of *Holarrhena antidysentrica* was found no significant changes in haematological, biochemical parameters and histopathological examinations.

KEYWORDS: *Holarrhena antidysentrica: Acute- toxicity: Sub-Acute- toxicity: Histopathology.*

1. INTRODUCTION

Holarrhena antidysentrica [*Family*: Apocynaceae] is commonly known as *Kutaj*, which is found in various parts of India. *Holarrhena* species is world- wide distributed from Africa to Asia. In relation to India it grows deciduous forests of Himalayan range.[1] *Holarrhena antidysentrica* have employed as a folk medicine remedy for inflammation and rheumatoid[2] amoebic dysentery, diarrhoea, asthma, branchopneumonia and anti-microbial activities.[3,4,5] *Holarrhena antidysentrica* has a role in larval growth inhibitor.[4] Methanolic extracts of *Holarrhena antidysentrica* seeds reported phytotoxic, antioxidant and cytotoxic activities.[7]

Holarrhena antidysentrica stem bark showed analgesic activity.[8] *Holarrhena antidysentrica* (seed, stem bark and leaves) major constituents are flavanods, terpinoids, steroidal alkaloid, phenolic acid, tannin, resin,saponin and ergostenol.[9] Alzheimer's disease tested five alkaloids viz Conessine, Isoconessimine, Conessimine, Conarrhimine and Conimine which is posses known inhibitor like AChE.[9]

The present investigation was conducted to toxicity study in detail *Holarrhena antidysentrica* in view of its medicinal importance in folklore medicine.

2. MATERIALS AND METHODS

2.1 Animal and Drug Administration

After approval of Institutional Animal Ethical Committee (IAEC), the present study was conducted in the Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi on inbred Albino mice (Swiss) 20-40g and Albino rats (Wistar Strain) 100-200g. They were kept in the departmental animal house in individual cages at an ambient temperature of 26 ± 3^0C and 60- 70% relative humidity with 12h:12h light: dark cycles. They had fee access to standard rodent pellet diet and drinking water (Kinley) during the entire study period. The food was withdrawn 18h prior to experimentation, however, water was allowed *ad libitum.*

2.2.1 Plant Material

The *Holarrhena antidysentrica* stem bark was obtained from the Regional Research Centre (Ay) Patna. Bihar, India and identified in the Department of Dravy guna, IMS, BHU, Varanasi. A few mg of powdered drug was warmed with Chloral hydrate, washed and mounted in glycerine. A few mg of powder was cleared in 4% KOH, washed and mounted in glycerine. A few mg of powder was washed in plain water, a drop of KI –solution was added and mounted. Camera Lucida drawings were done for the salient features of the drug. The voucher specimens have been preserved.

2.2.2 Extraction

Dried powdered (1kg) *Holarrhena antidysentrica* stem bark were extracted by ethanol, and concentrated in a steam bath to a final yield of 110.0g (11.0% w/w).Chemical tests showed the presence of steroids, alkaloids and flavonoids.

2.3.1 Acute-Toxicity Studies on Mice

Albino mice (Swiss) weighing 20-40 g were divided into 7 groups. These animals were fasted 18 h prior to the experimentation. Both the test and control groups were received in a same volume of drug or vehicle control as per body weight. Experiments were conducted as per OECD guidelines-423(Acute-Oral Toxicity-Single Dose).[10] The each group contains equal male and female mice (5 male + 5 female) were given graded doses of *H.antidysentrica* stem bark (10, 100, 200, 500, 1000 & 2000 mg / Kg, p.o.). Group –I received double distilled water as control. The animals were kept in observation for 96 h upto 14 days for any gross behavioural changes and mortality. The animals were observed for symptoms viz. writhing pilo-erection, salivation fur, lacrimation, convulsion, hyperreactivity, etc continuously for the first 4h after dosing. The numbers of survival were noted after 24h.These animals were then maintained and observed daily for 14 days for further any toxicity. Complete postmortem was done on all survivors or if any animal found dead or moribund condition during the study period. Histopathological examination was performed on all collected tissues of individual animals.

2.3.2. Sub-Acute Toxicity on Rats

Albino rats (Wistar strain) weighing 100-200g were divided into 4 groups. The each group contains equal male and female (6M + 6F=12), was treated with *H.antidysentrica* at the dose level of 200, 100and 50 mg/kg daily for 28 days. Group I received double distilled water in same ratio served as control (vehicle). The mortality rate, behavioral changes, if any was recorded during the experimentation. The body weight of animals, measured food and water were recorded weekly upto 28 days. Investigation of all animals in each group for the blood haematology (RBC, Hb, Prothrombine time, W.B.C., TC, DC, MCV, MCH, MCHC) and blood biochemistry (Blood glucose, SGOT, SGPT, Serum creatinine) on 14^{th} day and 28^{th} day. All animals in each group have been sacrificed on 30^{th} day the following vital organs *viz*., liver, kidney, lungs, spleen, ovaries, testes, stomach and intestine were separated, weighed for histopathological investigation of toxicity of the drugs, if any.

2.4 Statistical Analysis

All the data was analyzed by student's t-test followed by ANOVA.

3. RESULTS AND DISCUSSION

3.1Acute-Toxicity Studies on Mice

The animal treated with 2000, 1000 & 500 mg/kg, p.o. showed dull, writhing and 30% mortality recorded within 96 h. The other groups showed no mortality. After postmortem, histopathological examination was performed, actual route cause of mortality is higher exposure of dose.

3.2 Sub-Acute-Toxicity Studies on Rats

H.antidysentrica shows no significant effect in the blood haematology (RBC, Hb, Prothrombine time, W.B.C., TC, DC, MCV, MCH, MCHC), blood biochemistry (Blood glucose, SGOT, SGPT, Serum creatinine) body weight, weight of vital organs in comparison to control. The histological characters also showed no abnormal features in tissues studied. It is concluded that there is no specific pathological change detected in slides prepared in above said dose studied.

H.antidysentrica, apart from divers uses in folk medicine, has recently been shown to possess anti- inflammatory, analgesic and antioxidant properties.[8,9] The acute and sub-acute toxicity studies indicate that *H.antidysentrica* have a significant margin of safety in mice and rats.

Table 1: The body wt. of animals (g) treated with *H.antidysenterica.*Values are mean ±SE Figures in parentheses indicate number of animals used.

Group & Dose (mg/kg, p.o.)	Initial wt. [g]	1st week	2nd week	3rd week	4th week
Control (vehicle)	131.0 ± 5.27 (12)	148.0 ± 5.12 (12)	160.0 ± 4.47 (12)	154.0 ± 2.91 (12)	164.0 ± 9.72 (12)
H.antidysentrica 200	120.0 ± 3.46 (12)	138.0 ± 4.80 (12)	141.0 ± 6.47 (12)	128.0 ± 7.19 (12)	128.0 ± 6.47 (12)
100	132.0 ± 3.66 (12)	146.0 ± 3.01 (12)	156.0 ± 5.41 (12)	164.0 ±1.25 (12)	179.0 ± 5.56 (12)
50	122.0 ± 5.62 (12)	142.0 ± 5.47 (12)	146.0 ± 6.53 (12)	150.0 ±1.82 (12)	167.0 ± 5.14 (12)

Table 2: Sub acute toxicity: Food (g) and water (ml) consumption weekly treated with *H.antidysenterica*. Values are mean ± S.E. Figure in parentheses denotes the number of animals used.

Group & Dose (mg/kg, p.o.)	Food Consumption (Weekly)				Water Consumption (Weekly)			
	I	II	III	IV	I	II	III	IV
Control (Vehicle)	27.14 ± 1.54 (12)	34.28 ± 3.08 (12)	52.85 ± 6.18 (12)	58.57 ± 4.03 (12)	59.57 ± 3.41 (12)	55.71 ± 5.51 (12)	160.0 ± 9.24 (12)	151.42 ± 6.03 (12)
H.antidysentrica 200	32.85 ± 5.69 (12)	37.14 ± 3.01 (12)	50.0 ± 10.35 (12)	42.85 ± 6.54 (12)	55.02 ± 3.27 (12)	60.71 ± 3.92 (12)	161.42 ± 5.61 (12)	155.71 ± 11.60 (12)
100	32.85 ± 9.08 (12)	34.28 ± 4.02 (12)	41.42 ± 4.03 (12)	51.42 ± 1.69 (12)	59.28 ± 3.34 (12)	66.42 ± 4.25 (12)	157.85 ± 6.05 (12)	142.85 ± 12.54 (12)
50	37.14 ± 11.65 (12)	31.43 ± 1.19 (12)	58.57 ± 9.42 (12)	61.42 ± 4.79 (12)	57.85 ± 4.17 (12)	57.42 ± 1.62 (12)	150.71 ± 6.73 (12)	148.57 ± 6.31 (12)

Table 3: Sub-Acute Toxicity: Investigations of hematology of rat blood treated with *H.antisenterica* on 28 th day. Values are mean ± S. E. Figure in parentheses denoted the number of animals used.

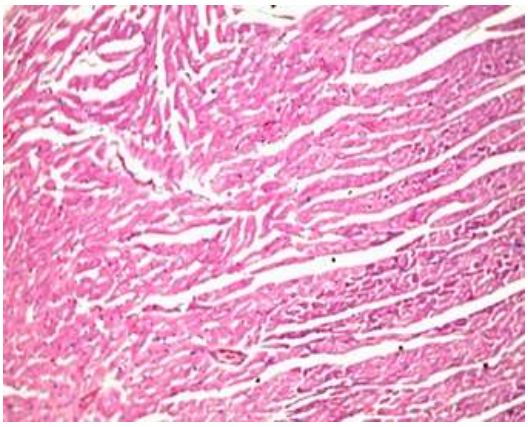
Groups & Dose (mg/kg p. o.)	Hb% g/dl	WBC 10^3 /mm^3	RBC 10^6 /mm^3	DC %				
				L	N	M	E	B
Control (Vehicle)	19.87±1.23 (12)	6800.00±245.64 (12)	6.56±031 (12)	62.8±2.73 (12)	32.00±2.28 (12)	1.40±0.60 (12)	3.4±0.60 (12)	0.40±0.24 (12)
*H.antidysentrica*200	17.20±0.96 (12)	6800.00±243.56 (12)	6.40±0.29 (12)	63.0±1.67 (12)	31.2±2.11 (12)	0.80±0.80 (12)	4.60±1.21 (12)	0.40±0.24 (12)
100	18.55±1.05 (12)	6450.00±197.00 (12)	4.70±0.70 (12)	62.4±2.21 (12)	31.4±1.07 (12)	0.80±0.80 (12)	4.60±0.68 (12)	1.0±0.44 (12)
50	19.70±0.41 (12)	6910.00±48.12 (12)	6.50±0.35 (12)	60.60±2.25 (12)	32.10±2.09 (12)	1.60±1.16 (12)	4.40±0.57 (12)	1.0±0.31 (12)

Table 4: Sub-Acute Toxicity: Investigations of Blood Biochemistry of rat blood (serum) treated with *H.antidysenterica* on 28th day. Values are mean ± S.E.Figure in parentheses denoted the number of animals used.

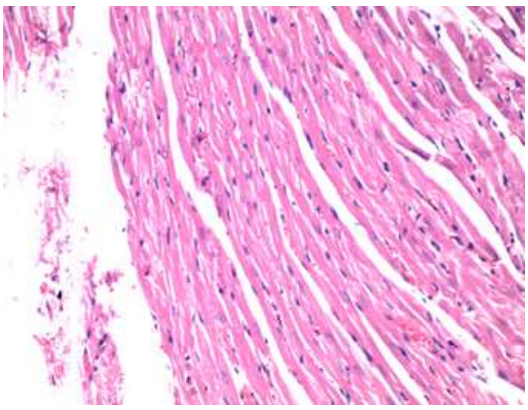
Groups & Dose mg/kg of b wt.	Total Bilirubin mg/dl	SGOT U/l	SGPT U/l	Albumin g/dl	Serum Creatinine mg/dl	Alkaline Phospatase U / l	Total Protein g/dl
Control (vehicle)	0.94 ± 0.16 (12)	114.2 ± 1.26 (12)	36.0 ± 5.30 (12)	3.67 ±0.22 (12)	0.52 ± 0.02 (12)	131.75 ± 7.66 (12)	6.91 ±0.33 (12)
H.antidysentrica 200	0.65 ± 0.27 (12)	142.4 ± 9.41 (12)	37.6 ± 4.61 (12)	4.12± 0.16 (12)	0.54 ± 0.02 (12)	177.4 ± 40.77 (12)	7.56± 0.31 (12)
100	0.45 ± 0.05 (12)	112.8 ±20.41 (12)	35.75 ±1.74 (12)	3.78 ± 0.12 (12)	0.52 ± 0.05 (5)	138.6 ± 30.13 (12)	6.52 ±0.27 (12)
50	0.71 ± 0.15 (12)	128.25±15.70 (12)	41.75 ±1.60 (12)	3.72 ± 0.08 (12)	0.55± 0.02 (12)	176.0 ± 32.20 (12)	7.05 ±0.30 (12)

Table 5: Sub-Acute Toxicity: The Weight of Vital Organ [g] treated with *H.antidysenterica* on 28th day. Values are weight in [g] mean ± S.E. Figure in parentheses denoted the number of animals used.

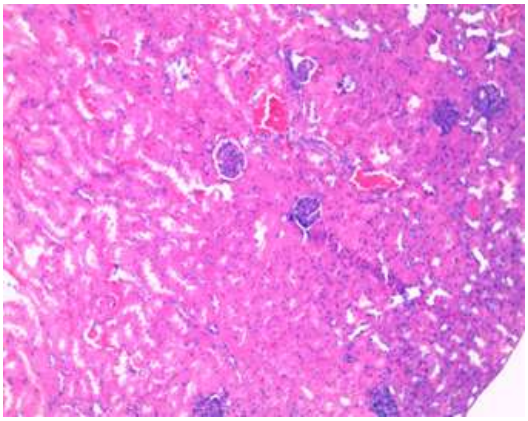
Groups & Dose mg/kg, p.o.	Heart	Kidney	Adrenal	Spleen	Liver
Control (Vehicle)	0.56±0.02 (12)	1.16±0.07 (12)	0.040 ± 0.004 (12)	0.36±0.03 (12)	4.98±0.34 (12)
H.antidysentrica 200	0.55±0.02 (12)	0.98±0.04 (12)	0.038±0.003 (12)	0.33±0.02 (12)	4.15±0.25 (12)
100	0.55±0.03 (12)	1.13±0.07 (12)	0.046±0.006 (12)	0.37±0.02 (12)	4.78±0.59 (12)
50	0.53±0.008 (12)	1.08±0.05 (12)	0.039±0.007 (12)	0.30±0.008 (12)	4.47±0.35 (12)



Heart Control



Heart High Dose [200mg/kg]



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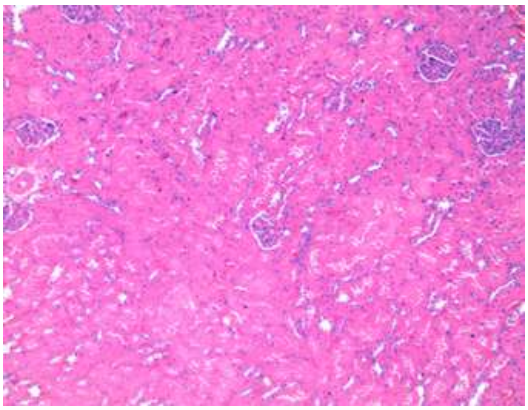
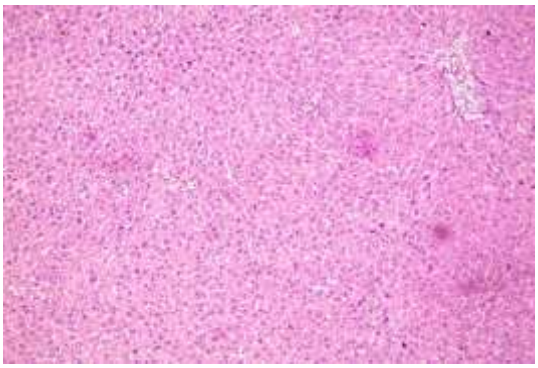
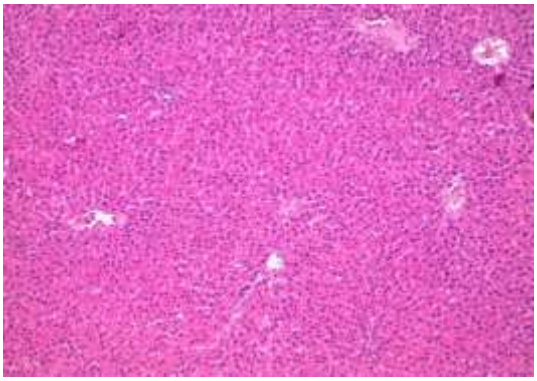


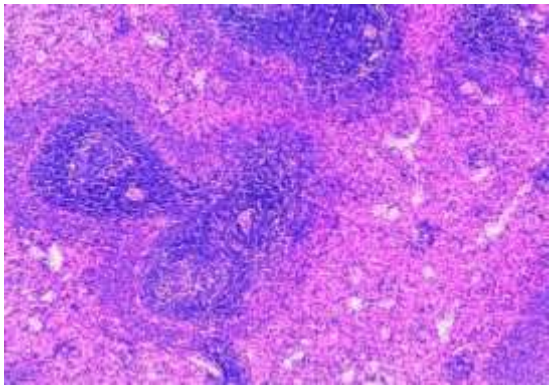
Kidney High dose[200mg/kg]



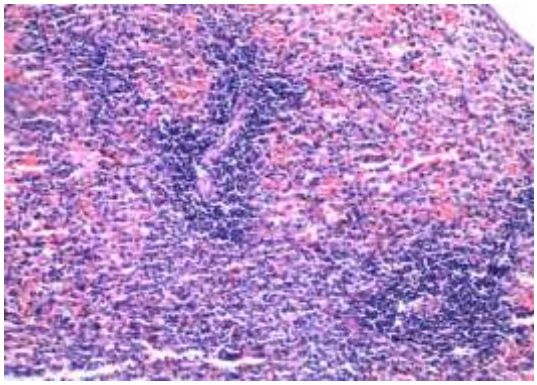
Liver Control



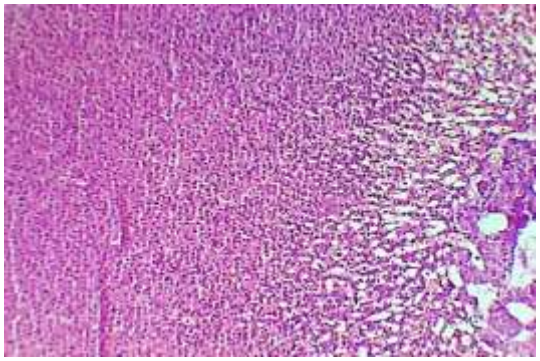
Liver High Dose[200mg/kg]



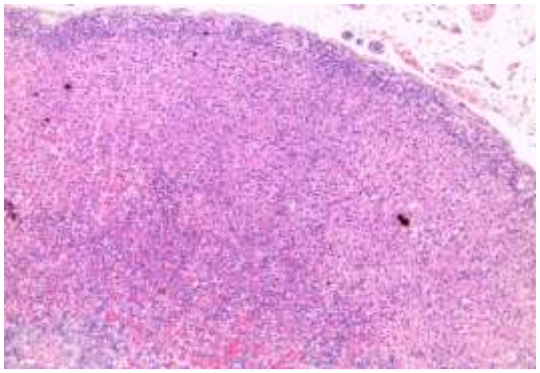
Spleen Control



Spleen High Dose[200mg/kg]



Adrenal Control



Adrenal High Dose [200mg/kg]

CONCLUSION

Acute toxicity of *H.antidysentrica* has safe upto the doses of 200 mg/kg and caused no mortality and normal behavior. The results of Sub-acute toxicity reveal that *H.antidysentrica* shows no significant effect in the blood haematology (RBC, Hb, Prothrombine time, W.B.C., TC, DC, MCV, MCH, MCHC), blood biochemistry (Blood glucose, SGOT, SGPT, Serum creatinine) body weight, weight of vital organs in comparison to control. *H.antidysentrica* is rich in alkaloids and flavanoids. Pure isolates of active principles need testing toward identifying amoebic dysentery, diarrhoea and immunomodulatory drug therapy for bronchial asthma.

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