INTERNATIONAL JOURNAL OF PHYTOTHERAPY RESEARCH
ISSN 2278 – 5701

Original Research Article

TOXICOLOGICAL STUDY OF RANDIA DUMETORUM LINN SEEDS IN WISTAR ALBINO RATS

Sanjaya Kumar Y.R*, Saketh Ram T, Thamizh Selvam N, Sushant S Parekar, Venugopalan T N and Sudarsanan Nair PK

National Research Institute for Panchakarma, Cheruthuruthy, Thrissur, Kerala

ABSTRACT:
Randia dumetorum (Retz.) Lam seeds were evaluated for safety in experimental animals. In acute toxicity study, the test drug was administered at dose of 5000 mg /kg body weight in Wistar rats and the animals were observed for mortality and signs of toxicity. In sub acute toxicity study, the test drug was administered at doses 200 to 2000 mg /kg body weight to Wistar rats for a period of 14 days. The test drug did not show any Signs of toxicity and mortality upon single exposure to the test drug. Though SGOT and SGPT showed transient increase, the values were normalized at the end of 14 days. During sub acute toxicity study, 25% and 50% mortality was observed in AD (Average Dose) and HD (High Dose) groups respectively. Significant decrease was observed in body weight gain and percent feed intake in AD and HD group as compared to control group. Hematological study revealed significant decrease in hemoglobin levels and relative decrease in packed cell volume and total red cells count. Biochemical studies revealed increase in the levels of SGOT, SGPT and decrease in creatinine levels as compared to the control group. Histopathological examination revealed no major morphological changes in the vital organs. It could be concluded from the study that the death observed during the study might be due to progressive gastric dilatation and the same must be the reason for elevated levels of liver enzyme.
Key words: Randia dumetorum, toxicity, hematology, serology, histopathology

INTRODUCTION:

Randia dumietorum (Retz.) Lam. / *Catunaregum spinos*a (Thunb.) Tirvengadum. / *Xeromphis* spinos (Thunb.) Keay, a thorny shrub belongs to family Rubiaceae and is seen in tropical and subtropical regions.

Ayurvedic Pharmacopoeia of India enlists the following vernacular names for Madanaphala: Sanskrit-Madani; Assamese-Maen ; Bengali-Mainaphal, English-Emetic nut; Gujrati -Mindhal, Hindi Manphal; Kannada-Mangarikai, Karigidda/Madanaphala Kashmiri-Madanfal; Malayalam- Malamkarakka; Marathi-Gal/Galphala, Oriya Maena, Madana; Punjabi-Mindhal, Tamil -Marukkarai; Telugu -Mranga Kaya, Monga Kaya ; Urdu -Mainphal [1].

Chardana, panda, nata, pinditaka, karhata, maruvaka, shalyaka, vishapushpaka are other important Sanskrit synonyms for the drug [2].

Being widely used in Ayurveda from 4 B.C onwards, the plant finds mention in Carakasamhita under asthapanopaga, anuvasanopaga, vamanadravya and phalini dravya varga. Susrutasamhita too mentions the same under aragvadhadivarga, mushkakadi, urdhvabhagahara groups [3].

Madanakalpa a chapter dedicated to Randia dumetorum proclaims that “madanaphala” is the best and safe emetic drug available for therapeutic purposes. The text recommends

ISSN 2278 – 5701

very specific time, method of harvesting the fruits and further processing them before employing for therapeutic purposes [4].

The drug is claimed as a medicinal use for piles, dysentry, asthma, jaundice, diarrhea and gonorrhea and as an emetic agent [5]. Ripe fruit contains glycosides, Randioside A, mollisidial triterpenoid glycosides and randianin, six saponins dumetoronius A to F. A hemolytic triterpenoid saponin that is Randianin has been isolated from fruit of *Randia dumentorum* [6].

MATERIALS AND METHODS

Animals

Wistar albino rats (male and female) were used in the trial. The rats were procured from Small Animal Breeding Station, Veterinary College, Mannuthy, Thrissur, India. After a period of quarantine, rats were caged individually with free access to pelleted feed and water.

Test drug

Randia dumetorum seeds were obtained from local market and authenticated. The seeds were grinded into fine powder and stored in airtight containers.

Ethical clearance

The present trial was proposed during the meeting of Institutional animal Ethics Committee (IAEC) held at National Research Institute for Panchakarma, Cheruthuruthy, Thrissur, Kerala.

Acute Toxicity study

The test was carried out as per OECD guideline 423[7]. The emetic dose of the test drug in humans is 3- 6 grams [1]. In acute study animals were dosed with the test drug equivalent to 6 grams of human dose. A total of 6 rats were used in the study. Initially 3 female rats were administered with 5000 mg of test drug (equivalent to 6 grams of human dose) once orally and the animals were observed for mortality for a period of 72 hours for mortality and for signs of toxicity over a period of 14 days. The procedure was repeated at the same dose with 3 male rats.

Sub acute toxicity study

A total of forty eight rats were divided into four groups, each containing six male and 6 female rats. The test was carried out in Wistar albino rats according to OECD guidelines No.407 [8]. Based on the findings of acute study, wherein serum creatinine levels were persistently high, the rats in the sub acute study were dosed with the test drug equivalent to 3000 mg of human dose and two dose levels below it. The test drug was administered at doses 200 (Therapeutic dose group), 1000 (Average dose group) and 2000 (High dose group) mg/kg once daily for a period of 14 days. Distilled water was administered to the control group. Mortality and signs of toxicity were recorded for a period of 14 days.

Individual body weights of animals were recorded initially and at weekly intervals till day 14. Feed consumption and body weight gain of individual animals were recorded at weekly intervals. Blood samples were collected from all the animals through retro orbital puncture under ether anesthesia on 15th day and animals were sacrificed.

Necropsy was performed to analyze the macroscopic changes in the major internal organs. Vital organs were collected, weighed and preserved in formalin for histopathology.

ISSN 2278 – 5701

Statistical analysis

The statistical difference between the control and test groups were calculated by means of analysis of variance followed by Dunnet's test with minimal level of significance set at $P \leq 0.05$. The results were expressed as Mean ± SEM.

RESULTS

The test drug upon single exposure at dose of 5000 mg/kg did not produce deaths or signs of toxicity in any of the animals during the study. Though there were slight increase in packed cell volume and total red blood cell count, the same was statistically not significant (Table 1). There was significant increase in levels of SGOT ($P<0.05$) and SGPT ($P<0.01$) at 7th day of the study, the same was found to be near initial levels by 15 days. The creatinine levels were also found to be at statistically higher levels at 7th day ($P<0.01$) and 15th day ($P<0.05$) (Table 2).

During sub acute toxicity study, AD group showed 25% mortality and HD group showed 50% mortality, but no mortality was recorded in TD group. The main signs of toxicity included restlessness, excitement, salivation in the beginning followed by dullness and death.

Significant ($P<0.05$) decrease in body weight gain was observed in AD ($P<0.05$) and HD ($P<0.01$) groups during 1 week of the study. Weekly gain in body weight was further decreased significantly in AD ($P<0.01$) and HD ($P<0.001$) groups during 2nd week as compared to Vehicle control group (VC) (Table 4). Correspondingly, significant reduction in percentage feed intake was observed during 1 and 2 weeks of the study in AD and HD group as compared to control (Table 4).

Hemoglobin levels were decreased significantly ($P<0.05$) in AD and HD groups (Table 5).

SGOT levels were significantly ($P<0.05$) increased in HD group. Whereas SGPT levels were found to be significantly higher in AD ($P<0.05$) and HD ($P<0.01$).Serum glucose levels were found to be significantly ($P<0.05$) reduced in HD group (Table 6).

There was a significant ($P<0.05$) increase in stomach weight of rats in HD group as compared to control (Table 7). Macroscopic examination during necropsy revealed distension of abdomen, bloating of intestine and hyperemia of liver (Fig. 1)

Histopathology examinations of vital organs did not reveal major changes indicative of tissue damage owing to test drug administration (Fig. 2 – 5). Histopathology of kidneys did not show any abnormalities and decrease in serum creatinine AD and HD groups could not be concluded.

DISCUSSION

Randia dumetorum seed powder was found to be safe up to dose of 5000mg /Kg body weight in rats during acute toxicity study. Increased PCV and TRC observed during the study might be due to hemoconcentration. Normalization of serum levels of SGOT and SGPT at the end of 15 days after initial rise might be an indication of absence of hepatotoxicity of the test drug. Noorani and Kale have demonstrated the hepatoprotective activity of methanolic extract of R.dumetorum fruit at doses 100 and 200 mg /kg body

ISSN 2278 – 5701

weight in chronic alcohol treated rats[9]. Significant increase in the serum creatinine levels during 7th day post test drug administration and presence of the same at significant levels might be due to presence of nephrotoxic saponins.

Mortality of the animals in AD and HD groups during sub acute toxicity study suggest that the drug might be toxic at respective doses. Decreased feed intake during the study might be due to stomach dilatation and the same might be the reason for loss of appetite, decreased body weight and death in some animals.

Decrease in Hemoglobin concentration in AD and HD groups might be due the presence of hemolytic saponin, Randianin[10]. Decrease in serum glucose level observed in HD group must be due to antidiabetic activity of the test drug[11].

The hepatic lesion is common in spontaneous and experimental cases of gastric dilation reflecting in a smooth to severe elevation of the serum concentrations of transaminases. The extrinsic vascular congestion and intrinsic gastric ischemia due to the stomach dilatation can reduce the systems hepatic endothelial reticulum capacity. Enhanced levels of SGOT and SGPT observed might be due to gastric dilatation upon test drug administration. The progressive gastric dilatation causes venous congestion of the vascularization adjacent to stomach and compressive action on the portal system and provokes portal hypertension. This consequently elevates the levels of glutamic pyruvic transaminase (GPT), as well as provoked tendency to enhance the levels of glutamic oxaloacetic transaminase[12].

Table 1. Effect of Randia dumetorum on hematological parameters during acute study.

	HB (g%)	TLC ($\times 10^{3)}$	Poly (%)	Lymph (%)	PCV (%)	TRC ($\times 10^{6}$)	Platelets ($\times 10^{5}$)
0 Day	12.60 ± 0.22	5.41 ± 0.27	29.33 ± 1.26	70.67 ± 1.26	31.00 ± 3.10	3.02 ± 0.24	2.32 ± 0.05
7th Day	13.22 ± 0.23	5.16 ± 0.35	27.17 ± 1.25	72.83 ± 1.25	36.67 ± 0.76	3.59 ± 0.17	2.29 ± 0.08
15th Day	12.75 ± 0.34	4.87 ± 0.40	26.67 ± 2.01	73.33 ± 2.01	36.50 ± 0.85	3.41 ± 0.20	2.26 ± 0.09

(Average of 6 Values)

ISSN 2278 – 5701

Table 2. Effect of Randia dumetorum on Biochemical parameters during acute toxicity study.

	Glucose	Total Protein	SGOT	SGPT	Creatinine	BUN
0 Day	79.33 ± 4.33	6.20 ± 0.24	111.67 ± 2.55	39.33 ± 2.46	0.83 ± 0.03	17.17 ± 1.35
7^{th} Day	75.17 ± 4.33	6.10 ± 0.21	123.33 * ± 1.23	57.00** ± 4.75	1.32 ** ± 0.04	19.33 ± 0.92
15^{th} Day	82.83 ± 4.44	6.13 ± 0.15	113.00 ± 4.46	44.33 ± 2.70	1.20*± 0.04	17.33 ± 0.49

* P≤0.5, ** P≤0.01, (Average of 6 Values)

Table 3. Effect of Randia dumetorum on Weekly % body weight gain (in grams) as compared to day 1 during sub acute toxicity study.

	C	T	A	H
0 - 1	2.34 ± 1.056	-1.96 ± 1.29	-6.140* ± 2.20	-8.66** ± 3.36
0 - 2	5.10 ± 1.38	-1.11 ± 1.13	-6.91** ± 2.34	-10.50** ± 3.25

* P≤0.5, ** P≤0.05 (Average of 6 Values)

Table 4. Effect of Randia dumetorum on Weekly % feed intake (in grams) during Sub acute Toxicity study.

	C	T	A	H
0 - 1	33.54 ± 1.46	30.83 ± 2.21	24.35* ± 4.15	21.77** ± 0.67
0 - 2	30.89 ± 1.405	28.63 ± 1.10	23.79** ± 0.85	20.85** ± 1.26

* P≤0.5, ** P≤0.05 (Average of 6 Values)

Table 5. Effect of Randia dumetorum on hematological parameters during sub acute toxicity study.

	HB (g%)	TLC ($\times 10^{3}$)	Poly (%)	Lymph (%)	PCV (%)	RBC ($\times 10^{6}$)	Platelets ($\times 10^{5}$)
Control	14.60 ± 0.48	7.93 ± 0.59	37.33 ± 2.25	62.67 ± 2.25	43.17 ± 1.83	4.20 ± 0.29	4.03 ± 0.36
TD	13.67 ± 0.31	7.83± 0.58	42.67 ± 4.21	57.33 ± 4.21	39.00 ± 1.91	4.35 ± 0.32	3.06 ± 0.20
AD	12.60* ± 0.70	8.00± 0.22	39.17 ± 2.04	60.83 ± 2.04	37.67 ± 1.82	4.1 ± 0.12	4.57 ± 0.42
HD	12.55* ± 0.70	7.10 ± 0.51	39.00 ± 1.59	61.00 ± 1.59	37.71 ± 1.35	3.75 ± 0.15	5.04 ± 0.49

* P≤0.05 (Average of 6 Values)

ISSN 2278 – 5701

Table 6. Effect of Randia dumetorum on biochemical parameters during sub acute toxicity study.

	Glucose (mg%)	Total Protein (g%)	SGOT (IU/L)	SGPT (IU/L)	Creatinine (mg%)	Prothrombin time (Sec.)
Control	73.50 ± 5.39	6.40 ± 0.16	87.83 ± 8.17	34.83 ± 3.38	1.083 ± 0.04	14.17 ± 1.58
TD	73.50 ± 5.39	6.23 ± 0.20	93.83 ± 4.04	37.00 ± 4.10	0.9 ± 0.05	10.83 ± 0.75
AD	64.00 ± 4.19	5.62 ± 0.35	121.67* ± 5.94	53.00* ± 5.260	0.78** ± 0.05	13.17 ± 1.14
HD	57.67* ± 2.96	5.65 ± 0.21	160.17** ± 13.30	59.66** ± 6.58	0.82** ± 0.02	15.00 ± 1.29

* P≤0.05, ** P≤0.01 (Average of 6 Values)

Table 7. Effect of Randia dumetorum on relative organ weights (in % Body Wt) during sub acute toxicity study

	Heart	Lung	Liver	Spleen	Stomach	Kidneys	Testes	Ovary	Brain
C	0.84 ± 0.08	0.33 ± 0.02	0.63 ± 0.04	0.22 ± 0.02	0.62 ± 0.03	0.65 ± 0.03	0.96 ± 0.01	0.05 ± 0.00	0.84 ± 0.08
T	0.82 ± 0.05	0.35 ± 0.02	0.67 ± 0.03	0.25 ± 0.02	0.62 ± 0.04	0.71 ± 0.03	1.16 ± 0.02	0.06 ± 0.00	0.82 ± 0.05
A	0.87 ± 0.07	0.34 ± 0.01	0.67 ± 0.05	0.22 ± 0.02	0.68 ± 0.03	0.64 ± 0.03	1.11 ± 0.01	0.06 ± 0.00	0.87 ± 0.07
H	0.92 ± 0.05	0.34 ± 0.01	0.69 ± 0.07	0.24 ± 0.02	0.82** ± 0.03	0.74 ± 0.02	1.13 ± 0.06	0.05 ± 0.00	0.92 ± 0.05

**P≤0.01 (Average of 6 Values)

ISSN 2278 – 5701

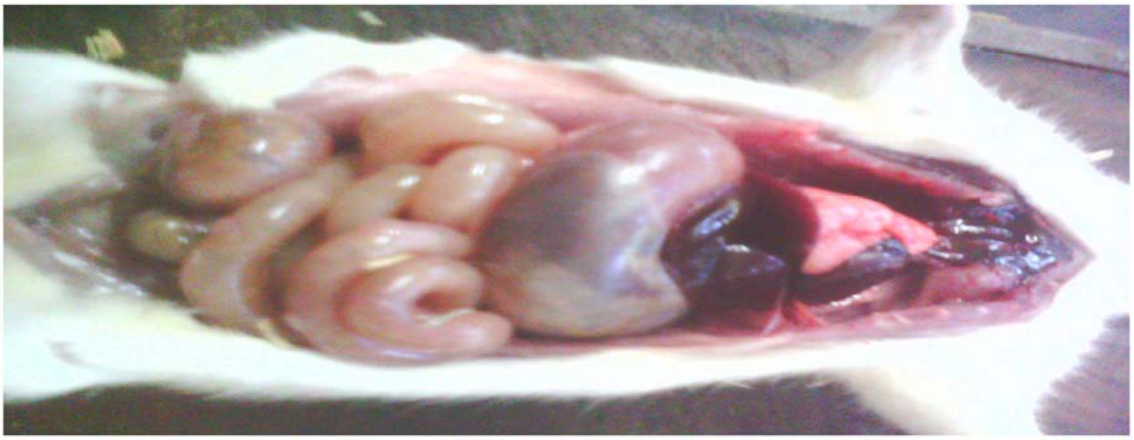

Figure. 1 Necropsy of a dead male rat showing gastric dilatation and bloated intestine.

ISSN 2278 – 5701

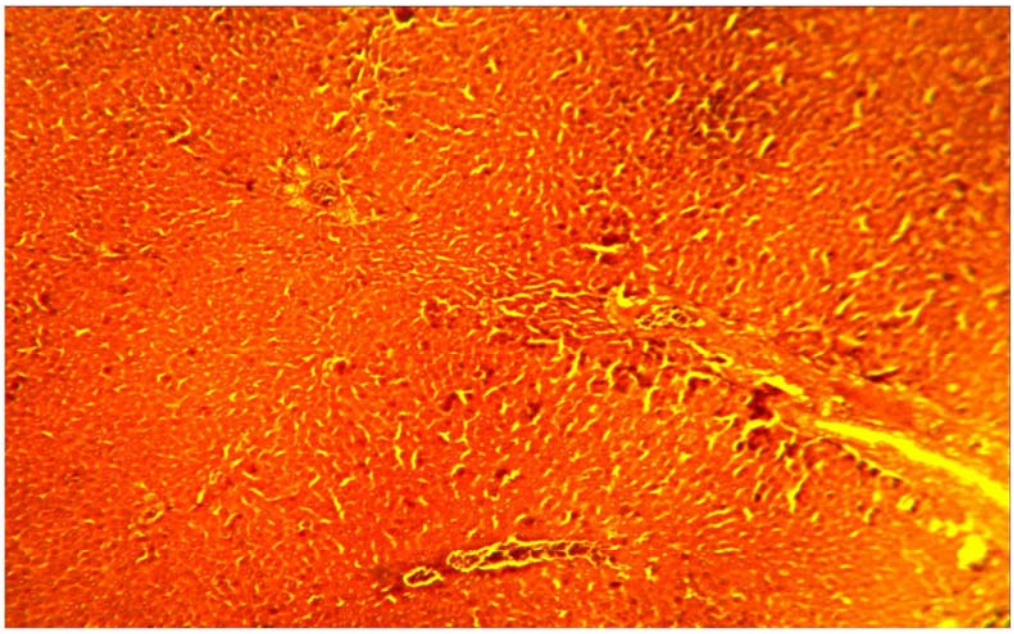

Figure. 2 Section of Liver of a female rat in high dose group showing normal portal triads and central venous system.

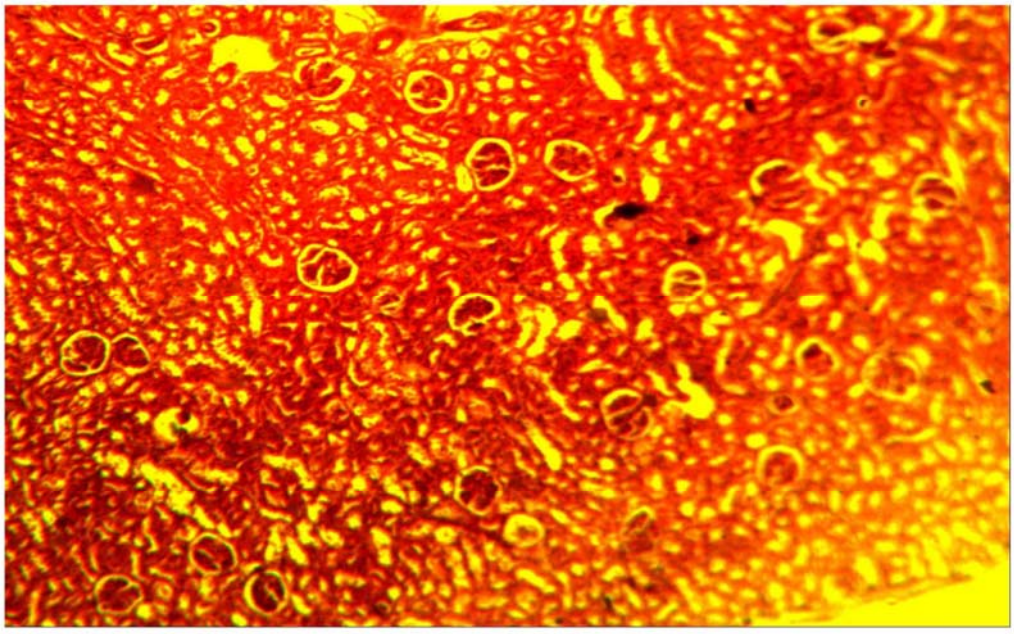

Figure. 3 Section of Kidney of a male rat in high dose group showing normal glomeruli with Bowman's capsule and normal renal tubules.

ISSN 2278 – 5701

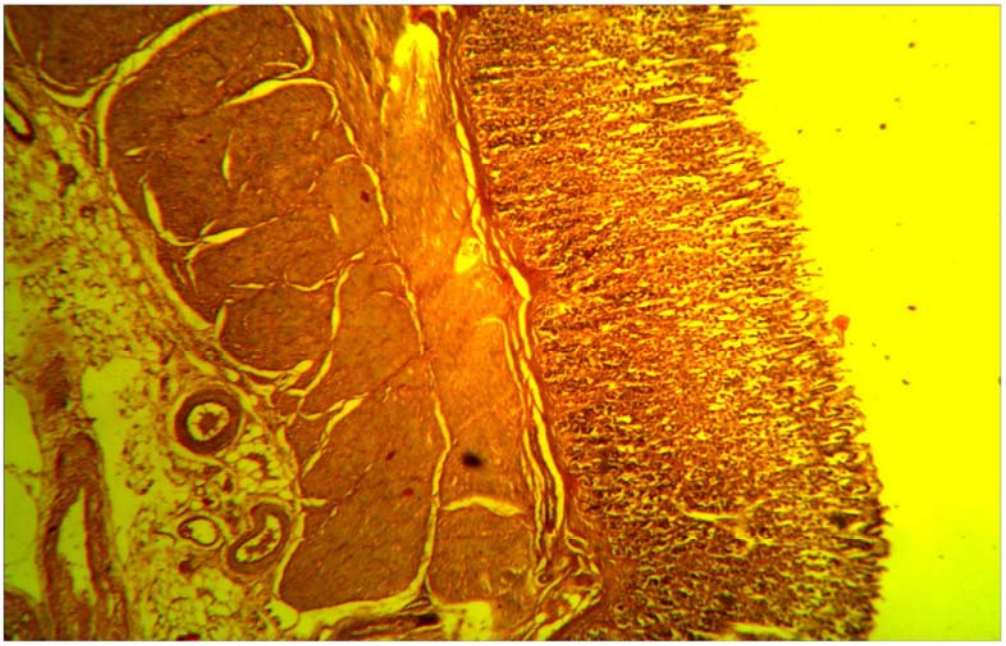

Figure. 4 Section of stomach of a male rat in high dose group showing normal mucosal glands lined by columnar epithelial cells and scattered lymphocytes and plasma cells in mucosal layer.

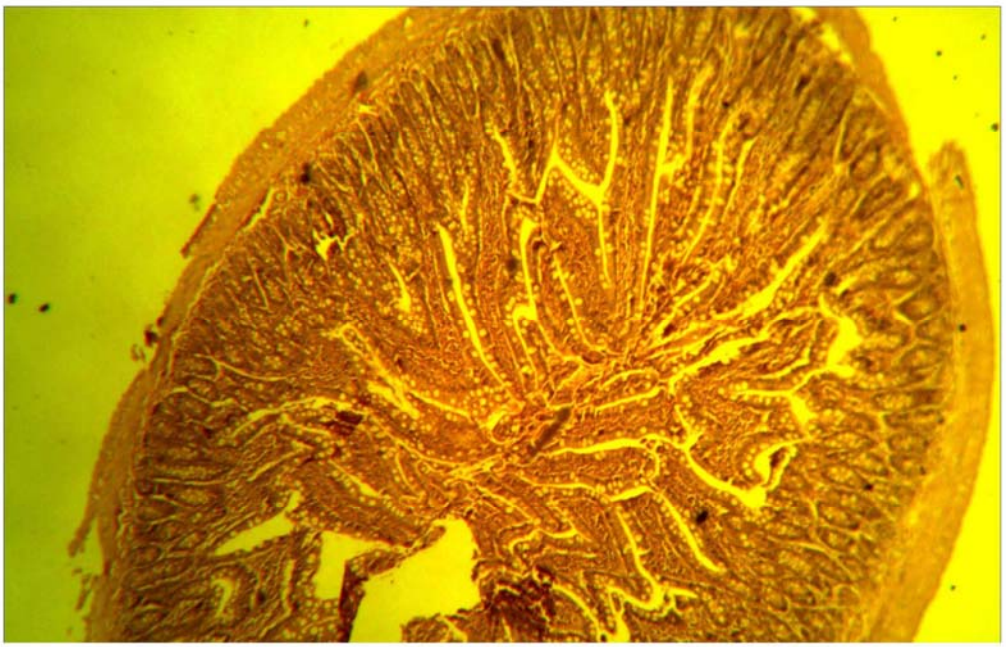

Figure. 5 Section of intestine of a female rat in high dose group showing normal villi lined by columnar epithelial cells and lymphocytic proliferation in the wall.

ISSN 2278 – 5701

CONCLUSION

During acute toxicity study, the test drug did not show any signs of toxicity and mortality upon single exposure to the test drug. Transient increase observed in SGOT and SGPT levels were normalized at the end of 14 days. During sub acute toxicity study, 25% and 50% mortality was observed in AD and HD groups, but no mortalities were recorded in TD group. Significant decrease was observed in body weight gain and percent feed intake in AD and HD group as compared to control group. Hematological study revealed significant decrease in hemoglobin levels and relative decrease in packed cell volume and total red cells count. Biochemical studies revealed increase in the levels of SGOT, SGPT and decrease in creatinine levels as compared to the control. Histopathological examination revealed no major morphological changes in the vital organs. These observations reaffirm the Ayurvedic conviction that the drug is "anapāyi (Carakasamhita. kalpasthana.1/13)" i.e. relatively non harmful as noted in Carakasamhita. However it is very important to note that, the drug needs to be properly collected, processed and stored appropriately as described earlier to ensure the safe therapeutic activity.

ACKNOWLEDGEMENT

Authors are thankful to Director General, CCRAS and staff members of National Research Institute for Panchakarma, Cheruthuruthy, and Thrissur for their cooperation.

REFERENCES

1. The Ayurvedic Pharmacopoeia of India 1st Edition , Part-1, Vol.-1. Dept of Indian System of Medicine and Homeopathy. New Delhi .2001: 84
2. K.C. Cunekar. Bhavaprakasa Nighantu (Indian Materia Medica). Chaukhamba Bharati
3. Academy. Varanasi. 2010:74
4. JLN Shastri. Illustrated Madanapala Nighatu (1st Edition Ed.). Varanasi: Chaukhamba
5. Orientalia Publishers. Varanasi 2010: 112.
6. Charaka Samihita. (Popular Edition ed.). Vol 1, Shree Gulabkunverba Ayurvedic Society
7. Jamnagar 1949:932.
8. Kirtikar K.R, Basu B.D Indian Medicinal Plants 2nd Ed. Volume 2. International book
9. distributers Dehradun India.1999:1760-1764.
10. Patel R.G ,Pathak N L, Rathod J D, Patel L.D , Bhatt N M Phytopharmacological properties of *Randia dumetorum* as a potential medicinal tree: An overview. Journal of Applied Pharmaceutical Science. 2011; 1(10): 24-26
11. OECD guidelines for the testing of chemicals Section 4 Health effects 2001 OECD, France.
12. OECD guidelines for the testing of chemicals Section 4 Health effects 2008 OECD, France.
13. Noorani A and Kale M K (2012). Pre treatment of albino Rats with methanolic fruit Extract of ***Randia dumetorum*** (***L.***) protects against alcohol Induced Liver Damage. The Korean Journal of Physiology and Pharmacology . Korean J Physiol Pharmacol. 2012 ;16 (2): 125 – 130.
14. Subramaniam S, Bokel M and Kraus W. A hemolytic saponin, Randianin from *Randia dumetorum.* Phytochemistry, 1989. 28(5): pp 1544-1546.
15. Mishra P.R, Panda P.K, Chowdary A.K & Panigrahi S. Anti diabetic and antihyperlipidaemic activity of *Randia dumetorum* . International Journal of Research in Pharmacy and Chemistry (IJRPC) 2012; 2(3): 552-559.
16. Santiago J RF and Kobayasi S. The effect of gastric dilatation in rats submitted to gasified water ingestion under the hepatic metabolic function Acta Cir. Bras. 2008; .23 (5):