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Ameliorative effect of *Tecomella undulata* stem bark on the cadmium chloride induced splenomegaly and its associated changes in wistar rats

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Abstract
Tecomella undulata (TU) was traditionally used in managing various disorders associated with spleen and liver dysfunctioning. In the present study, an aqueous extract of TU stem bark was prepared and evaluated for its protective effect on morphological, histological and haematological changes associated with splenomegaly induced by oral administration of cadmium chloride to Wistar rats. Animals were divided into eight groups having five animals in each group. Group I animals received standard food 50 g daily, Group II animals were administered with cadmium chloride with 50 g of food and Groups III-VIII was treated with TU extract (200 - 1200 mg/kg/day dose) along with group II treatment. The blood sample was taken from rats of all the groups by puncturing the heart tissue on 31st day for haematological studies, and then rats of the entire groups were killed. Spleen was examined for morphological and histological changes. Statistical analysis of the haematological, morphological and histological data of spleen among different groups revealed that cadmium chloride feeding caused splenomegaly, but when the aqueous extract of TU at the dose > 600 mg/kg/day was administered along with cadmium chloride, the spleen size and complete blood profile remains normal. Moreover, there was no significant change observed in the histology of spleen of TU extract treated animals of groups V-VIII.

Keywords: Tecomella undulate; splenomegaly; cadmium chloride; histology; haematology

Introduction
The spleen is the one of the most substantial organs in human and has been reported for its imperative role in hematopoiesis, filtering the blood, production of antibodies for the immune system and removal of invading infectious organisms [1]. There are many disorders associated with blood which may induce several changes in the morphology and physiological functioning of the spleen that leads to splenomegaly. Excessive physiological functioning, chronic inflammatory disorder, certain bacterial and parasitic systemic infections, extravagant medicines and haematological diseases have been considering the aggravating factor in splenomegaly [2-4]. The symptoms of splenomegaly were first appeared as inadequate breathing, gradual weight loss, indigestion and heaviness in the upper abdominal portion [5]. Its treatment is only underlying for the prevention of all aggravating conditions and its timely cure. However, yet there is no specific drug available for the treatment of splenomegaly. In lieu of this, the scientific community is now much more curious on controlling the physiological dysfunctioning of the spleen and also making unceasing efforts in the development of preventive drug therapy for splenomegaly. Resultantly, out of the various possible approaches, plant biodiversity probably could be treated as one of the most preferred hope in developing splenomegaly prevention therapy.
Tecomella undulata (TU) is a deciduous tree belongs to family bignoniaceae and well known by its vernacular name such as rohitaka in India. The plant has been well reported previously for its symbolic therapeutic effect in the treatment of spleen, liver and other abdominal disorders [6-8]. The rich chemical diversity of bark of TU further advocates its significant usage in indigenous system of medicine [9, 10]. Keeping its widespread therapeutic role in the backdrop, the present study was aimed to evaluate the effect of TU bark extract on cadmium chloride induced splenomegaly.

Materials and Methods

Experimental animals

Four-month-old healthy Wistar rats of either sex, weighing 140-150 g were procured from Guru Angad Dev University of Veterinary and Animal Sciences, Ludhiana, India. Rats were placed in propylene cages under standard housing conditions and fed standard rat pellet diet along with water ad-libitum. The entire study was conducted according to the prescribed protocol (BIS/COP/IAEC/01) of Institutional Animal Ethics Committee (Reg. No. 766/2007/2455/26/38/CPCSEA). The care of laboratory animals was taken as per the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India.

Table 1: Grouping of animals and treatment

Group*	Treatment
I	Standard food only 50 g
II	$CdCl_2$ 10 ml of 20 ppm solution mixed with 50 g of food
III	TU extract (200 mg/kg/day dose) + Group II treatment
IV	TU extract (400 mg/kg/day dose) + Group II treatment
V	TU extract (600 mg/kg/day dose) + Group II treatment
VI	TU extract (800 mg/kg/day dose) + Group II treatment
VII	TU extract (1000 mg/kg/day dose) + Group II treatment
VIII	TU extract (1200 mg/kg/day dose) + Group II treatment

*Each group contains 5 rats.

Plant collection and identification

The bark of TU was collected from the fields of Nohar, Hanuman garh (Rajasthan), in November. The taxonomical identification of bark was done by Dr. HB Singh, Scientist Incharge, National Institute of Science Communication and Information Resources, New Delhi. A voucher specimen of TU bark (NISCAIR/RHMD/Consult/2009-10/1326/128) was also deposited at the national herbarium of National Institute of Science Communication and Information Resources.

Preparation of aqueous extract of Tecomella undulata

Dried coarse powder TU bark (500 g) was extracted with distilled water at room temperature for 24 hours. The prepared extract was allowed to concentrate under reduced pressure using rota evaporator at the heating temperature below 70 ^{o}C to get 55 g of crude extract.

Acute toxicity studies

Twenty four Wistar-Albino rats were used in toxicity study under two trials having 12 animals on each trail. The animals were fasted for 12h before the study but were allowed water ad-libitum. In the first trial, four groups (n=3) were given normal saline as the control group and 10, 100 and 1000 mg/kg of the extract orally for the remaining three groups respectively. They were then observed for 24h for signs of toxicity or deaths. In the second trial, another four groups (n=3) were given normal saline, 2000, 4000 and 8000 mg/kg of extract orally for the remaining groups respectively and were observed for 24h for signs of toxicity or deaths. The acute toxicity study was carried out according to OECD guidelines. The median lethal dose (LD_{50}) was calculated.

Animals and experimental setup

Forty Wistar-Albino rats of either sex were weighed accurately and were used to the study the effects of aqueous extract of TU on the spleen of animals. They were kept in standard propylene cages at 25^{o}C under 12 h light/dark conditions in the animal room. They were fed on commercial rat's feeds and were given water ad-libitum. The animals were fasted from feeds for 12 h before the commencement of each experiment but were allowed water ad-libitum. Oral route was selected for testing of extract. Splenomegaly in animals was induced by oral administration of cadmium chloride 10 ml of 20 ppm solution. For testing of bark extract of TU the rats were divided into three large groups and received the treatment as mentioned in Table 1.

In each group, five rats were used. Morphological examinations of all the groups were performed by determining the average change in length and width of the spleen. The blood sample was taken from animals of all the groups by puncturing of heart tissue on 31st day for haematological study, and then rats of the entire groups were killed [11]. Before fixation in Bouin's fluid, the spleen was measured in each group for its length and width. For studying the internal structure of spleen tissues photomicrographs showing spleen cells were taken. For the photo-micrographic study, the fixed spleen was cut at 6µ and stained in Delafield's hematoxylin and eosin.

Statistical analysis

Size of spleen was expressed in centimeters (mean ± SEM). The data were statistically analyzed using ANOVA with multiple comparisons versus control group by Dennett's method. Values of $p<0.05$ or less were taken as significant [11].

Results and Discussions

Acute toxicity study

The mortality rates for 10, 100, 1000, 2000, 4000 and 8000 mg/kg of the extract was 0, 0, 0, 0, 51 and 100%. The LD_{50} was calculated as 3920 mg/kg body weight of Wistar rat.

Morphological study

Morphological studies of $CdCl_2$ treated rats indicate the significant increase in the length and width of the spleen (61% and 52% respectively) as compared to the controls. Meanwhile, increase in weight of spleen also occurred in both male and female rats. When $CdCl_2$ was administered along with TU extract and food, the spleen showed a minor difference in length and width, thus indicating the almost normal size and shape. TU extract exhibit dose-dependent effect on the morphology of spleen as shown in Table 2.

Table 2: Effect of cadmium chloride alone and in combination with extract of TU on the morphology of rat's spleen

Treatment	Length*(cm)±SEM	Width*(cm) ± SEM
Standard food only 50 g	16.651±0.9298	3.68±0.2124
$CdCl_2$ + food	26.861±1.374	7.02±0.1568
TU extract (200 mg/kg/day dose) + $CdCl_2$ + food	20.221±0.4928	4.32±0.3335
TU extract (400 mg/kg/day dose) + $CdCl_2$ + food	18.158±0.584	4.01±0.215
TU extract (600 mg/kg/day dose) + $CdCl_2$ + food	17.502±1.205	3.84±0.211
TU extract (800 mg/kg/day dose) + $CdCl_2$ + food	17.185±0.4928	3.32±0.3335
TU extract (1000 mg/kg/day dose) + $CdCl_2$ + food	16.984±0.214	3.05±0.254
TU extract (1200 mg/kg/day dose) + $CdCl_2$ + food	16.894±0.128	3.15±0.241

*Significant difference at 5% level of significance using Student 't' test.

Histological study

For histological study, fixed paraffin embedded spleen tissue was cut at 6 µ using microtome and was stained in Delafield's hematoxylin and eosin. The prepared slides were observed under an Olympus microscope fitted with a Sony SLR camera using the inbuilt software.

It was observed in histological studies that the control group (group I) of rats exhibited all characteristic features of normal spleen as shown in Figure 1a. The trabeculae were seen prominently, the white and red pulp was well differentiated with B and T lymphocytes in the spleen of control group rats. In comparison to the control group, the spleen of the $CdCl_2$ treated group of rats showed sinus congestion, with focal depopulation and hyperplasia of white matter. The pulp was organized, but at places, dead cells were seen. In the pulp, most of the cells were swollen as shown in Figure 1b. Similar, gross structures were also observed in the spleen of rats treated with 200 and 400 mg/kg/day of TU extract along with $CdCl_2$ and food. There was no significant change observed in histological characteristic features of the spleen in group V-VIII rats treated with TU extract (600, 800, 1000, 1200 mg/kg/day dose) along with $CdCl_2$ and food. There was no change observed in the shape of B & T lymphocytes, and neither hyperplasia nor any necrosis was seen in TU treated group at the dose > 600 mg/kg/day dose in comparison to $CdCl_2$ and food treated spleen as shown in Figure 1c, d.

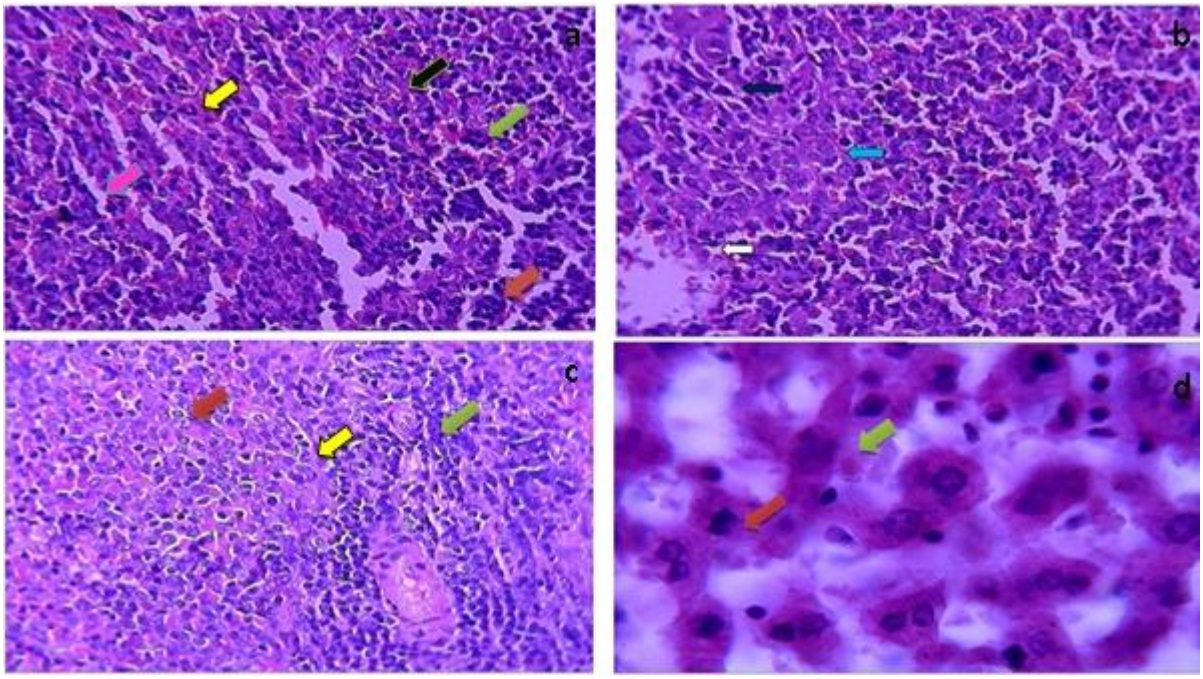

Fig 1: Histological studies of spleen

Haematological study

Haematological study of animals involves the determination of complete blood count (CBC). The haematological data of control, $CdCl_2$ treated and the drug-treated group was displayed in table 3. Control group (Group I) animals exhibited normal values of CBC. Following 31 days of $CdCl_2$ administration, $CdCl_2$ treated group (group II) showed significantly greater leukocyte counts than the control rat, but haemoglobin (Hb), Red blood cells (RBC), platelets and packed cell volume (PCV) values get significantly decreased compared to control group. There was no significant difference observed in the haematological profile of animals treated with TU extract 200 mg/kg/day dose and 400 mg/kg/day dose (group III and respectively) animals comparative to $CdCl_2$ treated animals. CBC profile of animals treated with TU extract >600mg/kg/day dose daily along with $CdCl_2$ and food was found to be normal or within the normal range. It was observed that there was no significant effect of $CdCl_2$ in animals treated with TU extract 600, 800, 1000 and 1200 mg/kg/day dose (group V, VI, VII, VIII respectively) and no leucopenia was observed in any of the animals of these groups. Thus it was concluded that TU aqueous extract might have some protective effect in the physiological functioning of the spleen.

Table 3: Summary of Haematological data

Group	Hb (gm%)	RBC ($X10^6$/mm^3)	Platelets (Lac/ml)	PCV (%)	Differential Count ($X10^3$/cu mm)				TLC (Per mm^3)
					Neutrophils	Lymphocytes	Monocytes	Eosinophils	
I	11.6±0.54	8.5±0.25	23.9±0.44	37.5±1.40	44.4±2.8	41.7±1.07	4.3±0.73	5.4±0.69	15420±352
II	7.3±0.70	5.3±0.33	16.7±0.24	26.0±2.3	74.4±2.5	83±2.73	9.2±1.22	9.3±0.62	19359±955
III	7.8±0.10	5.1±0.23	16.9±0.21	27.2±0.31	70.5±2.8	80±2.23	8.8±1.40	9.4±0.59	19012±845
IV	7.1±0.21	5.7±0.13	16.8±0.24	28.4±0.33	71.1±1.5	78±2.13	9.0±1.02	8.9±0.51	19108±784
V	9.9±0.12	8.0±0.23	20.4±0.22	31.4±0.24	50.1±1.4	49.5±2.33	5.8±1.22	5.01±0.15	15248±861
VI	10.5±0.20	8.0±0.13	21.4±0.32	32.4±0.22	51±1.01	50.1±2.01	5.4±1.12	4.91±0.25	15001±798
VII	10.1±0.12	8.3±0.22	22.7±0.58	34.4±1.3	51±2.94	48±2.75	5.5±0.82	4.89±0.45	15012±230
VIII	11.4±0.42	8.5±0.29	23.5±0.72	35.2±1.2	49±2.40	44±2.24	5.2±0.43	4.30±0.62	15890±550

Conclusion

When the spleen enlarges, it traps and stores an excessive number of blood cells and platelets, thereby reducing the number of RBC and platelets in the bloodstream. More the cells and platelets in the spleen traps, there will be a concurrent increase in the size of spleen. Eventually, the greatly enlarged spleen also traps normal red blood cells, destroying them along with abnormal ones. Also, excessive numbers of blood cells and platelet can clog the spleen, thus causing interfering with its function. The results showed that $CdCl_2$ administration causes splenomegaly and hyperplasia. When *Tecomella undulata* aqueous extract was administered along with $CdCl_2$ in the food, the size of the spleen remains normal and also no significant change observed in histology and haematological profile of animals. This suggests a protective effect of *Tecomella undulata* extract against splenomegaly.

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