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Toxicopathological evaluation of *Hymenocallis littoralis* and it's amelioration by *Achyranthes aspera* in Wistar rat

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Abstract
The present experiment was undertaken to investigate the toxic pathological, haemato-biochemical evaluation of *Hymenocallis littoralis* (Beach spider lily) in Wistar rats and amelioration by leaves powder of *Achyranthes aspera* was assessed. Fifty Wistar rats were procured for the study. Animals were acclimatized for a period of seven days and then divided into five groups of 10 rats (5 male+ 5 female) each. The group-I served as healthy control and was given standard feed and water *ad libitum*. The rats in group II were received 20% aq. extract of *Hymenocallis littoralis* (1000mg/kg body weight of animal) by an oral gavage, group III was administered an extract of *Hymenocallis littoralis*+ powder of *Achyranthes aspera* through feed. Group IV rats were fed with 1% of *Achyranthes aspera* powder and group V was administered both plants for 28 days. The present investigation last for the period of 28 days excluding acclimatization period. The blood and serum samples were drawn from retro-orbital plexus on 0, 14th day and 28th day from the rats in each group for the estimation of haemato-biochemical indices. There was significantly decreased in hematological parameters i.e., Hb, TEC counts whereas TLC were significantly increased. The biochemical parameters such as indicators of liver health (i.e., Mean of AST and ALT values) and indicators of the kidney (i.e., Mean Creatinine, Mean BUN) of rats of this group II were significantly increased. However, mean serum TP were significantly reduced in group II in comparison to other heathy control and plant control group rats. The feeding of *Achyranthes aspera* leaves powder against sub-acute *Hymenocallis littoralis* toxicity showed partial ameliorative effects on haemato-biochemical parameters.

Keywords: Hymenocallis littoralis, wistar rats, aqueous extract, haemato-biochemical indices, *Achyranthes aspera*

1. Introduction
Plant poisoning and fatalities due to exposure to poisonous plants are rare in industrialized nations. However, they are the major cause of livestock mortality in India, particularly in rural areas. Ruminants and companion animals are frequently poisoned by plants, which is the second most common class of toxicants after pesticides [6]. There are many poisonous plants and weeds widely distributed all over the world and when animals unintentionally consume them, they suffer from negative repercussions. The environment contains a lot of toxic plants, and they are frequently disregarded or simply overlooked until their effects, which might range from slight irritability or pain to quick mortality.

Among such poisonous plants, ornamental plants of the genus *Lilium* and *Hymenocallis*, are reported to be potentially nephrotoxic to the feline species. *Hymenocallis* spp. (Spider lily) mainly causes gastroenteritis in small ruminants [1]. *Hymenocallis littoralis* (Botanical name) which is commonly known as Beach Spider Lily belongs to the Family: *Amaryllidaceae*, which contains poisonous parts such as bulbs, leaves and flowers. Toxic constituents are *Amaryllidaceae* alkaloids such as lycorine and tazettine and other toxic plant alkaloids. A number of additional alkaloids have cholinergic, analgesic, hypotensive and cytotoxic actions among which lycorine is the main alkaloid having centrally emetic action, which is responsible for clinical symptoms [12]. In pets, chewing on or ingesting leaves generally causes mild gastrointestinal upset and ingestion of parts of the bulb may lead to more severe signs. *Amaryllidaceae* is a widely spread family all over the world containing about 90 genera and 1310 species.

The plant *Hymenocallis littoralis*, which is the main commercial species in Western India, it is around 60-70 cm tall with light green foliage. The sword-shaped leaves are broad in size

(4-5 cm) and the plant flowers throughout the year. It is used mainly as an ornamental plant, flowers used in perfumes, and bulbs contain lycorine and tazettine.

Herbal medicine is becoming more popular since it offers significant benefits without having a negative impact on health or productivity. Herbal remedies have a solid traditional or conceptual foundation and the potential to be effective pharmaceuticals for treating a variety of diseases due to their safety and efficacy [15].

Traditional medicinal plants, sometimes referred to as herbal remedies, botanical medicines, or phytomedicines, are generally composed of plant roots, stems, leaves, bark, seeds, and other edible parts that are cultivated in the ground and can be used to promote health and treat illnesses. *Hymenocallis littoralis* cause mainly nephrotoxicity as well as hepatotoxicity in animals. So, herbal medicinal plant which plays an important nephroprotective role i.e., *Achyranthes aspera* to ameliorate the toxic effect of *Hymenocallis littoralis* was selected and tried in the present study [20].

Achyranthes aspera possess multiple biological activities including diuretics, nephroprotective, hepatoprotective dermatological disorders, and gynecological disorders etc. The phytochemical constitutes of plants are alkaloids, saponins, glycosides, ecdysterone, cardiac glycosides, etc. are responsible for its nephroprotective effects. Hence, this plant was selected to conduct the experimental trial.

2. Materials and methods

This study was performed in laboratory animal house of Department of Pharmacology, College of Veterinary and Animal Sciences, Parbhani (MS).

Experimental Animals

Rats were kept in polypropylene cages (5 animals per cage), the room temperature was maintained at 20 ± 20C, humidity: 30-70%, photoperiod of 12 hrs light and 12 hrs dark period was given to animals. Corn cob was used as bedding material.

Hymenocallis littoralis plant collection and administration of rats

The test plant *Hymenocallis littoralis* plant was collected from the Department of Horticulture, Vasantrao Naik Marathwada Krishi Vidyapith campus, Parbhani, Maharashtra.

Collected leaves were washed, air dried and then, it was grind by using an electric grinder and 20% aqueous extract was obtained from these leaves powder of plant.

Leaves powder of *Achyranthes aspera*

Plant leaves of *Achyranthes aspera* were obtained from a nearby area of Parbhani (MS) which were dried, grind and powder was used throughout the experiment.

Experimental design

Fifty Wistar rats (Male+ Female) were divided into five groups. Group I was taken as the control group. The rats in group II were administered with 20% aqueous extract of *Hymenocallis littoralis* orally. Group III fed with 20% Aqueous extract of *Hymenocallis littoralis* + Powder of *Achyranthes aspera* plant @ 1% through feed (Preventive dose). Group IV was treated with powder of *Achyranthes aspera* plant and rats in group V were fed with Powder of *Achyranthes aspera* plant @ 1% through feed + 20% Aq. Extract of *Hymenocallis littoralis* orally (Therapeutic dose) for 28 days of the experiment excluding acclimatization period. The experiment was carried out after approval of the protocol by IAEC as per the guidelines of CPCSEA.

Hematology

Blood samples were collected from rats of each group from retroorbital plexus in EDTA vails on 0, 14th and 28th day of the trial. Hematological studies included the determination of hemoglobin (Hb) by acid haematin method, packed cell volume (PCV) by micro haematocrit method described by Jain (1986) [8], total erythrocyte counts and total leukocytes counts by haemocytometer method described by Sastry (1989) [14], differential leucocyte counts by method described by Weiss and Wardrop (2010) [23] and clotting time by capillary method described Benjamin (1985) [3].

Serum biochemistry

Blood samples collected from rats in each group on 0, 14th and 28th day of trial were allowed to clot in clot activator vial and centrifuged at 1000-1500 rpm for 20 minutes to separate and collected in serum collection vials.

Serum biochemicals estimations included Total Protein (gm/dL) by Varley (2005) [19], Blood glucose (mg/dl) by GOD POD method described by Trinder (1969) [18], serum AST (IU/L) and serum ALT (IU/L) by UK kinetic method described by Teitz (1976) [17], blood urea nitrogen (mg/dl) by Chaney and Marbach (1962) [15] and creatinine by Moss *et al.* (1975).

Statistical Analysis

The resulting data generated from various parameters will be statistically analyzed by using WASP (Anonyms, 2018 WASP version 2.0 http://www.ccari.res.in/wasp2.0/index.php.). Data were shown as mean± standard error.

3. Results

Hematological parameters

There was significantly decreased in mean values of hemoglobin, TEC in rats of group II whereas, TLC, neutrophil, and lymphocyte count were significantly inclined when compared with them on both 14th and 28th day of study. The mean values of PCV, clotting time were non-significantly vary in rats of all groups. There was non-significant change in body weight of rats in almost all groups. The mean values of TEC and TLC also significantly changed from control group animals. The mean values of total protein, blood glucose was significantly decreased as compare to control group rats. Haematological parameters of rats in group V showed mild improvement over rats in group II. The results of hematological studies have been presented in Table 1.

Table 1: Effect of subacute oral administration of aq. Extract of *Hymenocallis littoralis* on various haematological parameters in Wistar rats on 0, 14th and 28th day of study and protective effect of *A. aspera* leaves powder

Parameters	Groups	Intervals of study			P value
		0 day	14th day	28th day	
Haemoglobin (gm/dL)	I	12.96 ± 0.29	13.07 a ± 0.30	13.51 ab ± 0.34	NS
	II	13.00 ± 0.38	9.77c ± 0.44	8.93d ± 0.37	S
	III	12.59 ± 0.36	11.90b ± 0.42	11.55c ± 0.51	S
	IV	13.19 ± 0.23	13.52a ± 0.39	13.94a ± 0.25	NS
	V	12.06± 0.88	11.65b ± 0.09	12.68b ± 0.11	S
Packed Cell Volume (%)	I	38.40 ± 0.23	38.87 ± 0.28	39.68 ± 0.20	NS
	II	38.88 ± 1.02	39.23 ± 1.12	38.74 ± 0.96	NS
	III	40.49 ± 0.66	37.44 ± 0.95	39.02 ± 0.87	NS
	IV	39.28 ± 1.27	40.35 ± 0.94	39.64 ± 1.16	NS
	V	39.33± 0.69	40.40 ± 0.83	37.19 ± 1.12	NS
Total Erythrocyte Count (millions/cumm)	I	7.02 ± 0.07	7.14a ± 0.07	7.21a ± 0.05	NS
	II	6.89 ± 0.09	6.01c ± 0.16	5.75 c ± 0.19	S
	III	6.95 ± 0.05	6.67b ± 0.12	6.52 b ± 0.13	S
	IV	7.11 ± 0.05	7.06a ± 0.04	7.32 a ± 0.06	NS
	V	6.90 ± 0.08	6.70b ± 0.11	6.91a ± 0.09	S
Total Leucocyte Count (thousand/cumm)	I	9.27 ± 0.23	9.52bc ± 0.22	9.32b ± 0.26	NS
	II	9.42 ± 0.50	11.68a ± 0.20	13.02a ± 0.24	S
	III	9.65 ± 0.43	9.71bc ± 0.43	10.02b ± 0.33	S
	IV	8.98 ± 0.20	9.01c ± 0.15	9.28 b ± 0.25	NS
	V	9.16 ± 0.22	9.83 b ± 0.29	9.54b ± 0.29	S
Blood clotting time (sec)	I	53.13 ± 0.82	54.74±0.61	55.27±0.45	NS
	II	54.51 ± 0.96	57.69 ±1.16	59.10±1.21	NS
	III	53.83±0.81	55.86±0.98	57.60±0.77	NS
	IV	54.15 ± 0.88	54.83± 0.94	55.36±1.30	NS
	V	52.96±1.33	57.02±1.32	56.04±0.93	NS

Values indicate mean ± S.E
Non-significant (NS)=$P>0.05$, Significant (S)= $P<0.05$

Biochemical parameters

Investigation for biochemical parameters in rats of group II revealed that, indicators of liver health (i.e., Mean of AST, and ALT values) and indicators of the kidney (i.e., Mean BUN, Mean Creatinine) of rats of this group were significantly increased. Whereas, mean serum TP, and blood glucose values were significantly decreased when compared with values of rats in the healthy control and plant control group. The results of biochemical studies have been summarized in Table 2. Almost in all biochemical indices the rats in group V showed improvement.

Table 2: Effect of subacute oral administration of AQ. Extract of *Hymenocallis littoralis* on various biochemical parameters in Wistar rats on 0, 14th and 28th day of study and protective effect of *A. aspera* leaves powder

Parameters	Groups	Intervals of study			P value
		0 day	14th day	28th day	S
Total Protein (gm/dL)	I	6.97±0.10	a	b	NS
	II	6.68±0.10	4.97c±0.12	3.03d±0.13	S
	III	6.80±0.10	6.41b±1.95	6.29c±0.12	S
	IV	6.76±0.10	7.09a±0.07	7.29a±0.07	NS
	V	6.58±0.10	6.21b±0.20	6.85b±0.06	S
Blood glucose (gm/dL)	I	103.91±1.79	102.89±1.27	101.79a±1.24	NS
	II	102.62±1.39	98.77±0.72	c	S
	III	105.45±1.64	103.20±1.05	92.31b±1.32	S
	IV	101.53±1.59	100.88±1.17	102.26a±0.95	NS
	V	102.31±2.33	99.28±0.90	95.20b±1.33	S
Blood urea nitrogen (Mean ± S.E. mg/dL)	I	18.34±0.80	19.09c±0.74	19.60b±0.47	NS
	II	18.98±0.62	45.18a±2.01	61.96a±2.94	S
	III	20.30±0.28	19.38c±1.02	18.54b±1.12	S
	IV	19.09±0.46	18.78c±0.46	17.93b±0.69	NS
	V	20.17±0.47	27.60b±0.95	19.84b±0.62	S
Creatinine (Mean ± S.E. mg/dL)	I	0.67±0.14	0.75c±0.09	0.58b±0.06	NS
	II	0.85±0.10	4.10a±0.41	6.40a±0.49	S
	III	0.58±0.12	0.65c±0.13	0.62b±0.14	S
	IV	0.71±0.11	0.67c±0.09	0.43b±0.06	NS
	V	0.65±0.11	2.08b±0.25	0.76b±0.17	S
AST	I	74.28±0.50	73.41cd±0.46	72.78c±1.02	NS
	II	73.76±0.61	110.34a±2.63	131.28a±1.39	S
	III	74.02±0.69	77.37c±1.32	80.28b±1.68	S

(Mean ± S.E. IU/L)	IV	72.89±1.13	70.93d±1.05	68.16c±1.87	NS
	V	71.46±1.31	103.84b±1.82	84.77b±2.65	S
ALT (Mean ± S.E. IU/L)	I	32.68±0.35	33.19b±0.42	31.81bc±0.53	NS
	II	33.62±0.51	40.33a±0.65	46.06a±0.27	S
	III	32.08±0.64	31.87bc±1.01	30.94c±1.56	S
	IV	31.95±0.59	30.50c±0.68	29.91c±0.50	NS
	V	33.09±0.54	39.62a±1.26	33.88b±0.78	S

Values indicate mean ± S.E.
Non-significant (NS)=P>0.05, Significant (S)= p<0.05.

4. Discussion

The Hb concentration and total erythrocyte count in *Hymenocallis littoralis* intoxicated group i.e., group II was significantly reduced at both intervals of study (14th and 28th day). The hemoglobin value might have reduced due to alkaloids, organic acid, and hemolytic saponin present in *Hymenocallis littoralis* (*Amaryllidaceae*). The finding of hemolysis of RBCs noted by [23] and eryptosis which triggers cell membrane scrambling. This effect was partially due to the entry of extracellular Ca2+ [4] supports the present observation. The increase in total leucocyte counts of *Hymenocallis littoralis* extract toxicated rats might be due to granular leukocytes may be attributed to immunomodulation [2]. And also different phytochemicals such as phenols and flavonoids may be responsible for leukocytosis [13].

There was increment in lymphocyte and neutrophil counts at both 14th and 28th study interval in toxicated rats throughout the trial might be because of toxic effects of *Hymenocallis littoralis* [26] However, there was numerical elevated blood clotting time in toxicated rats.

Partial improvement in all haematological parameters of group V, might have been due to antioxidant properties due to different phytochemicals such as flavonoids, triterpenoids, alkaloids, tannins etc. present in the plant leaves which reduces the oxidative stress.

The decreased blood glucose level (hypoglycemia) in *Hymenocallis littoralis* treated rats. The reduction in blood glucose might be liver insufficiency which disturbed the normal glucose mechanism and to some extent anorexia also might be the culprit for hypoglycemia [10].

A significant decrease in serum total protein in rats of group II might be due to changes in protein and free amino acid metabolism in the liver. It could also be due to protein loss caused by a decrease in protein synthesis. In rats of group V, the mean total protein values were found to be improved than rats of group II and remained comparable with the control group value. Additionally, there was elevation in levels of BUN and Creatinine observed in rats of group II intoxicated with aq. extract of *Hymenocallis littoralis*.

The increase in BUN and Creatinine level in toxicated group. It has been recorded that *Lilies* causes renal failure in cats due to necrosis of renal tubular epithelial cells which may attributed to hike in Levels of BUN and *Creatinine values* [21]. Although the toxic principle(s) and mechanism of renal failure by *lilies* are unknown [16].

The significant incline in the mean values of AST and ALT in rats of group treated with aq. extract of *Hymenocallis littoralis* attributed to s It might be due to pronounced anorexia and associated hepatic stress had been evidenced by histopathological observations [7]. The elevated activity of these enzyme may reflect the impairment of the integrity in the liver parenchyma and bile canaliculi during excretion of high dose of the extract.

The remarkable decline in mean values of hepatic biomarkers (AST and ALT) in the rats of groups III and V at both the study intervals might have been notes due to hepatoprotective role of test plant *A. aspera* powder used in present trial. Mild significant improvement in serum total protein and blood glucose in rats of group V over aq. extract oh *Hymenocallis littoralis* toxicated birds indicates partial amelioration by leaves powder of *Achyranthes aspera.* This beneficial effect might be because of various phytochemicals and nutritional properties of plant *Achyranthes aspera*.

The partial reduction in elevated activities of BUN and Creatinine levels at the dose of 1000 mg/kg of extract of rats in group V, indicating that this nephroprotective effects of *Achyranthes aspera* could be attributed to its phytochemical phenolic compounds, predominantly flavonoids, triterpenoid and tannins, these compounds reduce the risk of nephrotoxicity [9].

5. Conclusions

From the findings of the present study, the following conclusions were made:

1. Administration of twenty percent aqueous extract of *Hymenocallis littoralis* @1000 mg/kg body weight through oral gavage daily for 28 days had primarily nephrotoxic effects as was evidenced by alterations in haemato-biochemical parameters in Wistar rats.
2. The extract of *Hymenocallis littoralis* caused gross and histopathological alterations in the vital organs, especially on kidneys of Wistar rats.
3. The feeding of 1% *Achyranthes aspera* powder through feed daily for 28 days resulted in a nephroprotective effect and thus ameliorate the toxic effects induced by *Hymenocallis littoralis* in Wistar rats.
4. More detail study would be helpful to establish the toxic effects of *Hymenocallis littoralis*.

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