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PHYTOCHEMICAL AND ACUTE TOXICITY STUDY ON *TINOSPORA TOMENTOSA* MIERS.

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Abstract : The stems of *Tinospora tomentosa* Miers. are used in Indian system of medicine. The present study deals with the preliminary qualitative phytochemical investigation and acute toxicity study on various extracts of the plant. The acute toxicity was studied in mice and rats in terms of 24 h LD_{50} of the methanol and aqueous extracts. These extracts were found to be non-toxic orally in doses up to 3.5 g/kg in both mice and rats.

Keywords: *Tinospora tomentosa* Miers. (Menispermaceae), phytochemical investigation, acute toxicity study

The plant *Tinospora tomentosa* Miers. (Menispermaceae) is a large deciduous climbing shrub mainly found in the tropical thickets of Bengal and almost throughout India, ascending to an altitude of 1000 meter. It is locally known as "Padmagulancha" and has long been used in Ayurvedic medicine. As per traditional use, the different parts like stems, leaves and roots of the plant are used as stomachic, bitter-tonic, anti-periodic, mild diuretic, emetic, anti-purgative, antipyretic, analgesic, anti-inflammatory, anti-diabetic, anti-leprotic, anti-gout (1-5). Some activities of some plants of this genus have been reported (6-10) The present study was undertaken to report the phytoconstituents present in successive extracts of the stem of the plant, to determine the ash values and extractive values of the dried stem and to find out the LD_{50} values of methanolic and aqueous extracts of the stem of the plant.

MATERIALS AND METHODS

Plant material

The plant was identified (Ref. No. CNH/I-I(53)/2004-Tech-I/885) by taxonomists of Botanical Survey of India, Shibpur, Howrah. After authentication, the fresh stems were collected in bulk from young matured plants at the rural areas of Howrah during July-August 2004 and washed, shade dried and milled into coarse powder by a mechanical grinder. The powder was passed through sieve number 40 (B.P standard) and used for further studies.

Preparation of extracts

The powdered plant material was extracted successively with redistilled, analytical grade petroleum ether (40-60°C), chloroform and methanol (procured from S.B. Fine Chem. Ltd., Mumbai and Merck, Mumbai) using Soxhlet apparatus. The solvents were removed under reduced pressure to obtain greenish-yellow (PE), brownish-black (CE) and reddish-brown (ME) colored solid residues (yield 2.9%, 3.4% and 14.1% w/w on dried plant material basis, respectively). Then, (with same plant material obtained after successive extraction with petroleum ether, chloroform and methanol) aqueous extract was prepared by decoction process using double distilled water. Then it was filtered, evaporated and dried under reduced pressure to give solid residue (AE) with yield of 22.5%, w/w on dried plant material basis. Phytochemical investigations were performed on all four extracts and LD_{50} study was done on methanol and aqueous extracts only, in mice and rats.

Preliminary phytochemical analysis

The extracts prepared in different solvents were taken and standard methods were used to detect the nature of phytoconstituents present in them (11-14).

Determination of total ash

About 2-3 g of accurately weighed powdered plant material was incinerated in a silica dish at a temperature not exceeding 450°C until free from carbon. It was then cooled and weighed. The % w/w

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Table 1. Qualitative phytochemical evaluation of the *Tinospora tomentosa* Miers. extracts

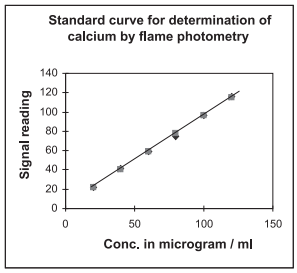
Constituents	Observations			
	PE	CE	ME	AE
Alkaloids	-	+	+	-
Flavonoids	-	-	+	-
Tannins	-	+	+	+
Saponins	-	-	-	+
Sugars	+	+	+	+
Protein	+	-	-	+
Organic acids	+	-	-	-
Glycoside	-	-	-	+

Table 2. Determination of ash and extractive values of the *Tinospora tomentosa* Miers.

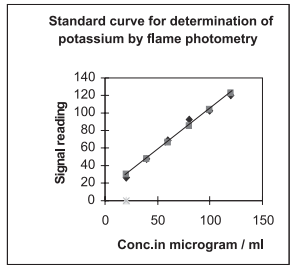
Ash value (%w/w)			Extractive value (%w/w)	
Total ash	Water soluble ash	Acid insoluble ash	Water soluble	Alcohol soluble
7.63 (0.1673)	4.92 (0.1320)	1.83 (0.1095)	20.50 (0.2234)	13.64 (0.2094)

Values in the parentheses are standard deviations.

Table 3. Determination of calcium, potassium and sodium levels of *Tinospora tomentosa* Miers.



R Square = 0.998693
S.E. of estimation = 1.460593



R Square = 0.988404
S.E. of estimation = 4.239834

Calcium	Potassium	Sodium
82.65	79.20	UD

Unit : µg/g of crude drug. UD : Undetectable.

of ash with reference to the air-dried plant material was calculated (15a).

Determination of etanol soluble extractive

Accurately weighed 5 g of air-dried powdered plant material was macerated with 100 mL of etanol of the specified strength in a closed flask for 24 h, shaking frequently during first 6 h and allowed to stand for 18 h. It was then filtered rapidly, taking precautions against loss of the solvent and 25 mL of the filtrate were evaporate to dryness in a tared flat-bottomed shallow dish and dried at 100°C to constant weight. The % w/w of etanol soluble extractive value was calculated with reference to the air-dried plant material (15b).

Determination of water soluble extractive

Procedure was the same as alcohol soluble extractive using chloroform and water (chloroform : water- 1: 399, v/v) instead of ethanol.

Determination of sodium, potassium and calcium

An accurately weighed amount of the ash of the plant was digested with 5 mL of 10% HCl. This was filtered through Whatman No. 41 filter paper and the residue was washed with hot water, cooled and made to volume. The sample solution was then compared in the flame photometer against standard solutions of NaCl, KCl and $CaCO_3$ containing the same amount of HCl. The concentrations of the

Table 4. Determination of LD_{50} values by Miller and Tainter method of the methanol and aqueous extracts of *Tinospora tomentosa* Miers. in mice

Group No.	Dose (mg/kg by *i.p.*)	Log dose	Dead/ total	% Dead	Corrected %*	Probit	LD_{50} value from graph (mg/kg)
ME-1	500	2.6989	0/10	0	2.5	3.04	1768.43
ME-2	1000	3.0000	1/10	10	10	3.72	
ME-3	1500	3.1760	2/10	20	20	4.16	
ME-4	2000	3.3000	5/10	50	50	5.00	
ME-5	2500	3.3979	7/10/	70	70	5.52	
ME-6	3000	3.4770	10/10	100	97.5	6.96	
AE-1	600	2.7700	0/10	0	2.5	3.04	1664.25
AE-2	1100	3.0400	1/10	10	10	3.72	
AE-3	1600	3.2000	3/10	30	30	4.48	
AE-4	2100	3.3200	5/10	50	50	5.00	
AE-5	2600	3.4100	9/10	90	90	6.28	
AE-6	3100	3.4900	10/10	100	97.5	6.96	

* Corrected formula: for 0% dead: 100 (0.25/n). fo 100% dead: 100 [(n-0.25)/n]
where n = number of animals in each group.

Table 5. Determination of LD_{50} values by Miller and Tainter method of the methanol and aqueous extracts of *Tinospora tomentosa* Miers. in rat

Group No.	Dose (mg/kg by *i.p.*)	Log dose	Dead/ total	% Dead	Corrected %*	Probit	LD_{50} value from graph (mg/kg)
ME-1	800	2.9000	0/10	0	2.5	3.04	1910.06
ME-2	1300	3.1100	1/10	10	10	3.72	
ME-3	1800	3.2500	3/10	30	30	4.48	
ME-4	2300	3.3600	6/10	60	60	5.25	
ME-5	2800	3.4400	8/10/	80	80	5.84	
ME-6	3300	3.5100	10/10	100	97.5	6.96	
AE-1	850	2.9200	0/10	0	2.5	3.04	2040.03
AE-2	1350	3.1300	1/10	10	10	3.72	
AE-3	1850	3.2600	2/10	20	20	4.16	
AE-4	2350	3.3700	5/10	50	50	5.00	
AE-5	2850	3.4500	8/10	80	80	5.84	
AE-6	3350	3.5200	10/10	100	97.5	6.96	

* Corrected formula: for 0% dead: 100 (0.25/n). fo 100% dead: 100 [(n-0.25)/n]
where n = number of animals in each group.

sodium, potassium and calcium ions were calculated by extrapolation method (16).

Ethical Clearance

Protocol used in this study for the use of animals was approved by the University Animal Ethical Committee.

Acute toxicity study

Swiss albino male mice (weighing 20-25 g) and Swiss albino male rats (weighing 110 – 130 g) were administered graded doses (300-3350 mg/kg of body weight) of methanol and aqueous extracts of the plant. After administration of the extracts (0.2 mL/mice, 0.5 mL/rat, intraperitoneally) the animals were observed for toxic effects after 24 h treatment. The toxicological effects were observed in terms of mortality and expressed as LD_{50}. The number of animals dying during the period was noted. The LD_{50} of the extracts were calculated by the method of Miller and Tainter (17, 18).

The methanolic and aqueous extracts were also administered orally in graded doses (500-3500 mg/kg body weight) in mice (0.5 mL/mouse) and rats (1.0 mL/rat) to test their oral toxicity. The solvent for preparing solution of methanolic and aqueous extract was sterile water for injection.

RESULTS AND DISCUSSION

The preliminary phytochemical tests performed were of qualitative type and the findings of the preliminary phytochemical investigations and the results of the LD_{50} study were depicted in the respective tables. From the phytochemical investigations it was observed that alkaloids, tannins and sugars were present in both CE and ME. Protein, organic acids and sugars were present in PE while saponins, tannins and sugars were present in AE. The total ash of the stem powder was 7.63% w/w. The water and alcohol soluble extractive values were 20.5 and 13.64% w/w, respectively. The amount of calcium and potassium present in the total ash were 82.65 and 79.2 µg/g of dried plant material, respectively. The aim of the total ash determination was to check the authenticity, purity and quality of the plant used for the study for reproducibility of the experimental results.

As the extracts were least toxic, so to get LD_{50} values, higher doses were administered intraperitoneally. From the acute toxicity study it was observed that the LD_{50} values of ME in mice and rats were found to be 1768.43 and 1910.06 mg/kg, intraperitoneally, respectively and of AE were found to be 1664.2 and 2040.03mg/kg, intraperitoneally, respectively. The extracts were found to be non-toxic orally in doses up to 3.5 g/kg body weight in mice and rat.

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