



Research Article

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## Evaluation of hypoglycaemic and wound healing activities of *Tiliacora acuminata*

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### ABSTRACT

Natural products particularly plant origin have played a vital role in prevention and mitigation of human diseases. The present study was carried to evaluate the anti-hyperglycemic and wound healing activity of the leaf extracts of *Tiliacora acuminata* (Lam) on rats, the aqueous leaf extract was found to produce significant reduction of blood glucose concentration between 2-4 h of administration in alloxan induced hyperglycaemic rats at tested dose levels. However, in normoglycaemic animals, the extract at 400mg/kg produced significant reduction of blood glucose between 2-4 h of administration. In the wound healing studies, the leaf juice was found to be more active than the extracts.

**Key words:** *Tiliacora acuminata*, Alloxan, Anti-hyperglycemic, Wound healing, Nitrofurazone.

### INTRODUCTION

*Tiliacora acuminata* (Lam) a member of Menispermaceae is a large evergreen, dioecious climbing shrub from a woody base. It is distributed throughout India. This plant has been used as an ingredient in many of the Ayurvedic preparations and the ethno medicinal uses of this plant include its use as an antidote for snake bite [1-2], the leaf or root paste is applied on the bitten area soon after bite [3], Juice from macerated leaves is applied to cuts in folks [4], crude and solvent extract of *T.acuminata* flowers shown highly activity against the larval form of *Culex quinquefasciatus* [5]. A new lactone [6], two alkaloids tiliareline and (+) N-methyl-tiliamosine were isolated from its leaves [7], Tannins, Steroids, Saponins, reducing sugars are reported from leaves [8] and an oil acuminated from seed have been isolated and characterized [9]. Several pharmacological and biological activities have been studied earlier. Anti-nociceptive and anti-diarrheal activities of ethanol leaf extract [10], antibacterial activity [11] and anti-inflammatory of different extracts of aerial parts [12] are reported.

### EXPERIMENTAL SECTION

Anti-hyperglycemic activity of the aqueous extract of the leaves was evaluated by using both normoglycaemic and alloxan induced hyperglycemic rats. The wound healing activity was assessed for both leaf juice and aqueous extract of the leaves on excised rats.

**Plant material**

*Tiliacora acuminata* leaves (2 kg) were collected from Parkal, Warangal and authenticated by Prof. V.S.Raju, Taxonomist, Kakatiya University and Warangal. A voucher specimen (MRM/06/2012) was deposited in the A.U. College of Pharmaceutical Sciences, Andhra University and Visakhapatnam. The material was shade dried and powdered.

**Preparation of the extract**

The dried powdered plant material (250 g) was refluxed with 500 ml of distilled water for 3 h, followed by filtration and concentration under vacuum, a brown sticky residue was obtained (yield: 15.63% w/v with respect to dried plant material).

**Wound healing evaluation of Aq.extract of the leaf of *T.acuminata* extract and fresh leaf juice of *T.acuminata***

The leaves of *T.acuminata* are being used by tribals for healing wounds. Hence, the author has made an attempt to study the wound healing activity on animal models to support the tribal claim. For testing the wound healing property, a method known as Excision wound model [13-14] was selected and All the experimental procedures were approved by Institutional animal ethical committee approval No. 1505/po/a/11/CPCSEA.

The selected Wistar rats were divided into four groups of six in each. The skin hair was removed by using a depilatory cream. Light incisions were made on the cleared surface by cutting the skin of the animals under mild ether anesthesia. The area of the wounds was measured (sq.mm) immediately by placing a transparent polythene graph paper over the wound and then tracing the area of the wound on it. This was taken as the initial wound area reading. All the test samples were applied topically. Group-I served as control. Group-II served as reference to which nitrofurazone (0.2 % w/w in simple ointment) was applied topically. Group-III animals were treated with the Aq. extract (10 %w/w in simple ointment I.P. and the Group-IV animals with the juice of the fresh leaves in a similar manner. All the test samples were applied twice daily. The wound area of each animal was measured on 1<sup>st</sup>, 4<sup>th</sup>, 8<sup>th</sup>, 11<sup>th</sup> and 14<sup>th</sup> day. The percentage healing was calculated from the days of measurements of wound area. The results were presented in Table 1.1 as Mean  $\pm$  S.E.M. Significance of difference between control and treated groups was determined using Student's *t*-test.

**Table-1.1 wound healing activity of the leaf Aq.extract and fresh leaf juice of *T. acuminata* in excised rats**

Group	Treatment	Percentage inhibition of wound on the day of study				
		1 st	4 th	8 th	11 th	14 th
I	Control	0	19.27 $\pm$ 3.26	46.87 $\pm$ 5.87	56.28 $\pm$ 2.48	62.73 $\pm$ 1.16
II	Nitrofurazone (0.2% w/w)	0	24.69 $\pm$ 7.84	57.35 $\pm$ 7.36	86.24 $\pm$ 0.82	95.16 $\pm$ 0.33*
III	Aq. extract of <i>T.acuminata</i> (10% w/w)	0	20.6 $\pm$ 5.68	48.24 $\pm$ 4.78	71.61 $\pm$ 2.91	87.12 $\pm$ 1.28*
IV	Leaves juice of <i>T.acuminata</i>	0	22.46 $\pm$ 6.75	57.26 $\pm$ 6.46	83.26 $\pm$ 1.83	94.24 $\pm$ 0.48*

Results expressed as Mean  $\pm$  SEM from six observations. \* *P* < 0.001 on 14<sup>th</sup> day of study.

**Anti-diabetic evaluation of the aqueous extract of *T.acuminata***

The anti-diabetic screening of the aqueous extract of the leaves of *T.acuminata* was studied on both alloxan induced diabetic rats and normoglycaemic rats.

**Anti-diabetic evaluation of the aqueous extract of *T.acuminata* using hyperglycaemic rats**

The acclimatized animals were kept fasting for 24 h with water *ad libitum* and injected intraperitoneally a dose of 120 mg/kg of alloxan monohydrate in normal saline. After one hour, the animals were provided feed *ad libitum*. The blood glucose level was checked before alloxanisation and 24 h after alloxanisation by withdrawing blood from the tip of the tail of each rat under mild ether anesthesia [15-16]. The blood glucose level was measured with haemoglucostrips supplied by M/s Pulsatum Health Care Pvt. Ltd., Bangalore with the help of a Pulsatum blood glucose monitor.

Animals were considered diabetic when the blood glucose level was raised beyond twice the value of normal. This condition was observed at the end of 48h after alloxanisation. The animals were segregated into four groups of six rats in each. Group-I served as control and received vehicle (2 ml/kg) through oral route. Group-II received Glibenclamide (2.5mg/kg). Group-III and IV received the extract at doses of 200 and 400 mg/kg in a similar manner. Blood samples were collected from each rat by cutting the tip of the tail under mild ether anesthesia. Blood

glucose level was estimated at 0 h, 1 h, 2 h, 4 h and 8 h respectively. The results were expressed as mean  $\pm$  S.E.M. in Table-1.2. Significance of difference between control and treated groups was determined using Student's *t*-test.

#### Anti-diabetic evaluation of the aqueous extract of *T.acuminata* of using normoglycaemic rats

The animals were fasted for 18 h, but were allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn from the tip of the tail of each rat under mild ether anesthesia and the blood glucose was estimated as above. The normal rats were then divided into three groups of six animals each. Group-I served as control and received vehicle (2 ml/kg) through oral route. Group-II received Glibenclamide (2.5 mg/kg). Group-III and IV received the extract at doses of 200 and 400 mg/kg in a similar manner. Blood glucose levels were measured after 1, 2, 4 and 8 h of administration of single dose of test samples. The results were expressed as mean  $\pm$  S.E.M. in Table-1.2. Significance of difference between control and treated groups was determined using Student's *t*-test.

**Table-1.2 Hypoglycaemic activity of aqueous extract of *T.acuminata* on blood glucose concentration in alloxan induced hyperglycaemic rats**

Group	Treatment	Dose	Blood glucose conc. (mg/dl.)				
			0h	1h	2h	4h	8h
I	0.5% w/v Sodium CMC (Vehicle)	2 ml/kg	295.83 $\pm$ 5.45	311.17 $\pm$ 7.8	321.83 $\pm$ 6.4	335.5 $\pm$ 6.52	352.83 $\pm$ 7.91
II	Glibenclamide	2.5 mg/kg	292.17 $\pm$ 5.16	183.17 $\pm$ 16.78* (37.31%)	137.0 $\pm$ 11.37* (53.11%)	103.0 $\pm$ 4.89* (64.75%)	74.83 $\pm$ 4.21* (74.39%)
III	Aq. extract of <i>T.acuminata</i>	200 mg/kg	300.67 $\pm$ 7.68	298.83 $\pm$ 6.89 (0.61%)	246.67 $\pm$ 8.87* (17.96%)	170.5 $\pm$ 12.84* (43.29%)	134.67 $\pm$ 9.24* (55.21%)
IV	Aq. extract of <i>T.acuminata</i>	400 mg/kg	311.67 $\pm$ 6.9	304.83 $\pm$ 7.27 (2.19%)	224.83 $\pm$ 5.63* (27.86%)	125.5 $\pm$ 9.72* (59.73%)	98.5 $\pm$ 9.0* (68.4%)

Results expressed as Mean  $\pm$  SEM from six observations.

**Table-1.3 Hypoglycaemic activity of aqueous extract of *T.acuminata* on blood glucose concentration in normoglycaemic rats**

Group	Treatment	Dose	Blood glucose conc. (mg/dl.)				
			0h	1h	2h	4h	8h
I	0.5% w/v Sodium CMC (Vehicle)	2 ml/kg	96.17 $\pm$ 3.14	98.0 $\pm$ 3.66	97.17 $\pm$ 2.65	96.17 $\pm$ 2.06	97.24 $\pm$ 2.11
II	Glibenclamide	2.5 mg/kg	98.67 $\pm$ 3.6	82.67 $\pm$ 2.76* (16.21%)	73.33 $\pm$ 2.56** (25.68%)	64.5 $\pm$ 1.29** (34.63%)	62.17 $\pm$ 0.68** (36.99%)
III	Aq. extract of <i>T.acuminata</i>	200 mg/kg	97.17 $\pm$ 3.16	95.25 $\pm$ 2.18 (1.97%)	88.38 $\pm$ 2.76 (9.04%)	79.13 $\pm$ 2.19** (18.56%)	71.23 $\pm$ 1.36** (26.69%)
IV	Aq. extract of <i>T.acuminata</i>	400 mg/kg	93.18 $\pm$ 2.59	90.29 $\pm$ 3.12 (3.1%)	78.23 $\pm$ 4.06 (16.04%)	71.83 $\pm$ 3.26** (22.91%)	68.29 $\pm$ 3.06** (26.71%)

Results expressed as Mean  $\pm$  SEM from six observations.

## RESULTS AND DISCUSSION

The studies on wound healing activity revealed that the nitrofurazone treated animals showed 95.19% healing on 14<sup>th</sup> day of study on the other hand, the extract treated group showed 87.13% healing and leaf juice treated groups exhibited 94.19% wound healing. The leaf juices caused significant reduction in the blood glucose levels in the rats. The extract was found to produce marked reduction in blood glucose concentration between 2-4 hours of administration in both alloxan induced hyperglycaemic and normoglycaemic rats at tested dose levels as depicted in Table-1.2 and 1.3 respectively. When compared with the reference control Glibenclamide, the extract caused noticeable reduction in the blood glucose level in both classes of animals except that the onset of action of Glibenclamide was noticed from the first one hour. The comparable effect of the *T.acuminata* with Glibenclamide was interesting and the constituents present in the extract may have similar activity as of Glibenclamide. This justifies the use of the plant in the folklore diabetic treatments. The results indicate that the extracts of the *T.acuminata* is endowed with effective wound healing and hypoglycaemic effects.

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