



## Evaluation of Diuretic activity from *Tylophora indica* leaves extracts

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### ABSTRACT

Aqueous and alcoholic extracts of *Tylophora indica* leaves were tested for diuretic activity in rats. The parameters studied on individual rat were body weight before and after test period, total urine volume, urine concentration of Na<sup>+</sup>, K<sup>-</sup> and Cl<sup>-</sup>. In the present study alcoholic and aqueous extracts of *Tylophora indica* leaves (100mg/kg of body weight) showed increase in urine volume, cation and anion excretion. Furosemide was used as reference diuretic.

**Keywords:** Diuretic activity, Furosemide, *Tylophora indica*

### INTRODUCTION

Diuretics are drugs that increase the rate of urine flow, sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations. Drug-induced diuresis is beneficial in many life-threatening disease conditions such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure, hypertension, and pregnancy toxemia [1]. Most diuretic drugs have the adverse effect on quality of life including impotence, fatigue, and weakness. Naturally occurring diuretics include caffeine in coffee, tea, and cola, which inhibit Na<sup>+</sup> reabsorption and alcohol in beer, wine and mixed drinks, which inhibit secretion of ADH [2, 3]. Although most of the diuretics proved to be very effective in promoting sodium excretion, all cause potassium loss and prompted the search for potassium sparing diuretic. Hence search for a new diuretic agent that retains therapeutic efficacy and yet devoid of potassium loss is justified [4]

*Tylophora indica* (Asclepiadaceae) is a branching climber, leaves ovate to orbicular, cordate – based.

It is used in the treatment of emetic, cathartic, expectorant, diaphoretic, asthma, whooping cough, dysentery. Phytochemical literature reveals the presence of tylophorin, alkaloids, tannins, saponins, flavonoids in *Tylophora indica* [5,6,7,8,9]. No systematic studies have been reported for its diuretic activity. Hence an effort has been made to establish the diuretic activity of aqueous and alcoholic extracts of *Tylophora indica*.

### MATERIALS AND METHODS

#### Plant collection

Fresh leaves of *Tylophora indica* were collected in the month of March from the district of Madurai in Tamilnadu. It was identified and authenticated. The voucher specimens were deposited at the college for future reference.

#### Preparation of extracts

200gms of dried and powdered leaves were extracted with alcohol in Soxhlet apparatus for 24 hours (3 cycles hour). A dark brownish green colored residue was obtained after concentrating the extract under reduced pressure (Yield – 6.2%). The aqueous extract was obtained by macerating 250gms of powdered *Tylophora indica* leaves with 5 liters of

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distilled water (72 hours). The extract was filtered and concentrated under reduced pressure to obtain a green colored residue (Yield – 5.2%).

### Experimental animals

In bred colony strains of Wistar rats of either sex weighing 150-250 g procured from the animal house were used for the study. The animals were maintained in polypropylene cages of standard dimensions at a temperature of  $28 \pm 1^\circ \text{C}$  and standard 12 hour : 12 hour day / night rhythm. The animals were fed with standard rodent pellet diet ( Hindustan Lever Ltd) and water *ad libitum*. Prior to the experiment the animals were acclimatized to the laboratory conditions.

### Preliminary phyto chemical analysis

The preliminary phytochemical analysis [10,11] were carried out to find out the phytoconstituents present in the crude extracts.

### Diuretic Activity

Male rats (wister albino strain) weighing 150 to 180gm were maintained under standard condition of temperature and humidity. The method of Lipschitz et al [12,13] was employed for the assessment of diuretic activity. The experimental protocols have been approved by the Institutional Animal Ethical Committee. Four groups of six rats in each and were fasted and deprived of water for eighteen hours prior to the experiment. The first group of animals serving as control, received normal saline(25ml/Kg,p.o.); the second group received furosemide (100mg/Kg,i.p.) in saline; the third, fourth groups received the Alcohol and Aqueous extract at the doses of 100 mg/Kg, respectively, in normal saline. Immediately after administration the

animals were placed in metabolic cages (2 per cage), specially designed to separate urine and feces, kept at room temperature of  $25 \pm 0.5^\circ \text{C}$  through out the experiment. The urine was collected in measuring cylinders up to 3 hrs after dosing. During this period, no food or water was made available to animals. The parameters taken for individual rat were body weight before and after test period, total concentration of  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  in the urine.  $\text{Na}^+$ ,  $\text{K}^+$  concentrations were measured by Flame photometry [14] and  $\text{Cl}^-$  concentration was estimated by titration [15] with silver nitrate solution(N/50)using three drop of 5% potassium chromate solution as indicator. Furosemide sodium salt was given by stomach tube. Optimal dose activity relation was found to be 20mg/Kg of furosemide per kg body weight in series of supportive experiments. Results are reported as mean  $\pm$  SD, the test of significance ( $p < 0.01$  and  $p < 0.05$ ) was stastically.

### Statistical analysis

All the results are expressed as mean  $\pm$  standard error. The data was analyzed statistically using ANOVA [16] at a probability level of  $P < 0.001$ .

## RESULTS AND DISCUSSION

The preliminary phyto chemical analysis showed the presence of Flavanoids, Glycosides, Saponins, Carbohydrates, Proteins and Aminoacids, Tannins, Terpenoids and Alkaloids in all the extracts (Table 1). Present study shows that the aqueous and alcoholic extracts of *Tylophora indica* leaves possess good diuretic activity. Urine volume, cation and anion excretion were increased,  $\text{Na}^+/\text{K}^+$  ratio

Table1. Preliminary Phytochemical test of *Tylophora indica* Alcohol, Aqueous extract.

| S.No. | Phytochemical tests     | Alcohol extract | Aqueous extract |
|-------|-------------------------|-----------------|-----------------|
| 1.    | Carbohydrates           | +               | +               |
| 2.    | Glycosides              | +               | +               |
| 3.    | Alkaloids               | +               | +               |
| 4.    | Saponins                | +               | +               |
| 5.    | Tannins                 | +               | +               |
| 6.    | Proteins and Aminoacids | +               | +               |
| 7.    | Flavonoids              | +               | +               |

Table 2. Showing effect of extracts of *Tylophora indica* on excretory parameters

| Treatment         | Dose mg/kg | No. of Rats used | Urine volume (ml) | Electrolyte Excretion |                     |               | Total Chloride $\mu$ Moles/kg |
|-------------------|------------|------------------|-------------------|-----------------------|---------------------|---------------|-------------------------------|
|                   |            |                  |                   | Na + $\mu$ Moles/kg   | Ka + $\mu$ Moles/kg | Na+/K + ratio |                               |
| Normal Saline     | 25ml/kg    | 6                | 2.2 $\pm$ 12      | 1987 $\pm$ 38         | 906 $\pm$ 31        | 2.193         | 631.28                        |
| Aqueous Extract   | 100ml/kg   | 6                | 4.1 $\pm$ 0.82    | 3062 $\pm$ 32*        | 1496 $\pm$ 501*     | 2.046         | 2010 $\pm$ 12                 |
| Alcoholic Extract | 100mg/kg   | 6                | 1.9 $\pm$ 0.30    | 2090 $\pm$ 40         | 1120 $\pm$ 12       | 1.866         | 2210 $\pm$ 80                 |
| Furosemide        | 100mg/kg   | 6                | 3.6 $\pm$ 0.36    | 2998 $\pm$ 04         | 1662 $\pm$ 312      | 1.550         | 26.90 $\pm$ 110               |

The values are expression of the mean standard error \* P<0.001 vs. control.

of 2.04 and 2.18 were obtained for aqueous and alcoholic extract respectively. The normal value for  $\text{Na}^+/\text{K}^+$  ratio is reported to be 2.05 – 2.83. The concentration of aldosterone is found to be dependent on  $\text{Na}^+/\text{K}^+$  ratio. If the  $\text{Na}^+/\text{K}^+$  ratio falls below the normal in plasma the aldosterone secretion will be decreased and if the ratio rises above the normal value the aldosterone secretion will be increased. Significant increase in  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  ion excretion was observed in aqueous and alcoholic extract treated animals but it was less than the furosemide control. Further studies are required to assess the medicinal value of leaves of *Tylophora*

*indica* as a potential diuretic agent (Table 2).

Diuretics relieve pulmonary congestion and peripheral edema. These agents are useful in reducing the syndrome of volume overload, decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure [17]. Thus, diuretics play an important role in hypertensive patients. In present study, we can demonstrate that ethanol, aqueous and chloroform extract may produce diuretic effect by increasing the excretion of Sodium, Potassium and Chloride. The control of plasma sodium is important in the regulation of blood volume and pressure; the control of

plasma potassium is required to maintain proper function of cardiac and skeletal muscles [18]. The regulation of Sodium, Potassium balance is also intimately related to renal control of acid-base balance. The Potassium loss that occurs with many diuretics may lead to hypokalemia. For this reason, generally potassium-sparing diuretics are recommended [19]. In present study chloroform and alcohol extracts showed elevated levels of Potassium in urine, which may increase risk of hypokalemia and hence its potassium sparing capacity has to be investigated. Active principles such as flavanoids, saponins and terpenoids are known to be responsible for diuretic activity [20,21,22]. Results of present investigation showed that ethanol is most effective in increasing urinary electrolyte concentration of all the ions i.e Sodium, Potassium and Chloride followed by chloroform and aqueous extracts while other extracts did not show significant increase in urinary electrolyte concentration.

A complex set of interrelationships exists among the cardiovascular system, the kidneys, the central nervous system ( $\text{Na}^+$ , appetite, thirst regulation) and the tissue capillary beds (distribution of extracellular fluid volume), so that perturbation at one of these sites can affect all the remaining sites. A primary law of the kidneys is that  $\text{Na}^+$  excretion is a steep function of mean arterial blood pressure (MABP) such that small increase in MABP cause marked increase in  $\text{Na}^+$  excretion [23]. One of the earliest strategies for the management of hypertension was to alter  $\text{Na}^+$  balance by restriction of salt in the diet. Diuretic agents having antihypertensive effects were used alone

and had greater efficacy than all other antihypertensive drugs.

In this study pharmacological evaluation of diuretic action of aqueous and alcoholic extracts of *Tylopora indica* were evaluated using furosemide under controlled laboratory condition. As diuretic therapy may lead to number of life threatening electrolytic disorder and toxicities, so safety profile studies are carried out following a sub chronic administration of extracts. This amplify the heterogenous array of diuretic curatives available for safe and effective treatment of edema and cardiovascular diseases [24].

## CONCLUSION

The extracts of *Tylophora indica* has diuretic effect supporting the ethnopharmacological use as diuretics. This effect may be explored in the use of the plant in the management of some cardiovascular diseases.

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