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*Research Article*

# Evaluation of Diuretic Activity of *Passiflora Nepalensis* in Rats

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**ABSTRACT**

This study was conducted to investigate the diuretic potential of aqueous methanolic extract of *Passiflora nepalensis* in rats. The extract at doses of 10, 30 50 and 100 mg/kg was administered intraperitoneally in acute diuretic model. Furosemide (10 mg/kg i.p) was used as standard drug. Total urine volume and urinary excretion of electrolytes were measured. Preliminary phytochemical tests were also performed using standard procedures. The extract exhibited a significant dose dependent diuretic effect at all the doses when compared to control group. Urinary excretion of sodium was significantly increased by the extract while a significant increase in potassium excretion was only observed at higher doses. Phytochemical analysis illustrated that the extract contained compounds such as alkaloids, saponins, tannins, flavonoids, cardiac glycosides and reducing sugars. It is conceivable therefore that the aqueous methanolic extract of *P. nepalensis* contain certain biologically active compounds that might be responsible for the diuretic activity.

**Key-words:** Diuretic, Furosemide, *Passiflora nepalensis*

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## INTRODUCTION

Diuretics are the drugs that promote urine production, sodium excretion and adjust volume of body fluids in various clinical situations. They have been considered an effective treatment for diseases like hypertension, cardiac failure, nephritic syndrome and pulmonary edema (Devi et al., 2010). Historically, these agents have been classified on a variety of on potassium excretion (potassium sparring diuretics; Katzung, 2003). Despite the abundance of these synthetic drugs, a significant proportion of the population of developing countries depends on traditional ideas such as efficiency (high ceiling diuretics), place of action (loop diuretics), structure (thiazide diuretics) and effects medicines for their health related needs. This is due to the fact that the efficacy of the modern synthetic drugs has been only 60%, and generally a combination of these drugs is required to achieve optimal therapeutic outcomes. Moreover, side effects from these medications have also raised serious medical concerns (Du and Chen, 2005). According to WHO, about 60% of the population in developed countries rely on traditional herbal medicine, and the percentage is much higher in the developing world (WHO, 2000). Traditional remedies, though effective for a number of diseases, require scientific evaluation to be used to their full extent. Since there has been no regulatory authority to evaluate the rational use of traditional medicines, hence mode of action of most of these traditional medicines remains yet to be elucidated (Tomassoni and Simone, 2001). Therefore, there is a need to determine the efficacy of these traditional herbal medicines.

The genus Passiflora consists of 500 species which are mostly found in warm and tropical regions. Passiflora comes from Latin word "Passio" that was first time discovered by Spanish discoverers in 1529 and was described as a symbol for "Passion of Christ" (Kinghorn, 2001; Dhawan et al., 2004). Passiflora was used widely in traditional medicine in East India, Mexico, Netherland, South America, Italia and Argentina. One of species of this genus named as Passiflora nepalensis (Passifloraceae) is more popular than its other species in Eastern India. Passiflora nepalensis is used in folklore medicine for treating hypertension and inflammation (Patel, 2009; Patel et al., 2009). Passiflora contains several compounds including alkaloids, phenols, glycosyl flavonoids and cyanogenic compounds (Dhawan et al., 2004; Patel, 2009; Patel et al., 2009).At present, there was no scientific study available on the diuretic activity of this plant; hence this study was aimed at exploring the therapeutic potential of aqueous methanolic extract of *Passiflora nepalensis* in diuresis.

## MATERIALS AND METHODS

### Chemicals and drugs

Standard grade methanol (Merck Chemical Co, Germany) and Furosemide (Aventis Pharma Ltd.) were used.

### Plant material and extraction

The whole plant of Passiflora nepalensis were collected in the month of October from the Eastern part of India (Sikkim Himalayas). The Herbarium Specimen (No-168) of plant was deposited in the department of Pharmacognosy and identified from Himalayan Pharmacy Institute, Majhitar. Aqueous methanolic (70:30) extract of P. nepalensis (whole plant) was prepared using cold maceration process (Yuchi et al., 2012). The extract was then air-dried to obtain a solid mass of percentage yield(16%).

### Animals

Both male and female Wister rats weighing 250 -350 g were used for the experiment. They were housed in standard environmental condition like, ambient temperature (25° ± 1° C), relative humidity (55±5%) and 12/12h light dark cycle. Animals had free access to standard pellet diet and water ad libitum. All animal experiments were carried out in accordance with the guidelines of CPCSEA. The institute animal ethical committee has given approval for conducting animal experiments (HPI/08/60/IAEC/0060).

### Diuretic test

Rats of either sex weighing (250-350 g) were assigned into six groups (n = 6). Group I served as control and received normal saline (10 mL/kg). The animals in group II were treated with Furosemide (10 mg/kg) that served as standard group. Group III and group IV received 10 mg/kg and 30 mg/kg of aqueous methanolic extract of *P. nepalensis* respectively. Animals in Group V and group VI were treated with 50 mg/kg and 100 mg/kg of the extract respectively. The animals were fasted overnight (16 h) prior to the test but with free excess to water only. Briefly,

after administration of various doses of the extract by intraperitoneally route, animals in each of these groups were placed in metabolic cages for 6 hours at a temperature of 25 ± 0.5 ºC. Volume of urine collected in diuretic chamber was measured after every hour for 6 consecutive hours. Then total volume of urine (mL) in 6 hours was calculated. Finally, urine volume was expressed as mL/100 g body weight. Urinary electrolytes concentration ($Na^+$ and $K^+$) were measured by flame photometer from the freshly obtained urine. Diuretic action and diuretic activity were also determined using formula: Diuretic action = Urine volume of treated group / urine volume of control group; Diuretic activity = diuretic action of treated group/diuretic action of standard group (Patel et al., 2010).

**Phytochemical analysis**

The extract was screened for various phytochemical constituents (alkaloids, saponins, tannins, flavonoids, terpenoids, cardiac glycosides, sugars) using standard methods (Khandelwal, 2006).

**Results**

The aqueous methanolic extract of *P. nepalensis* produced a significant dose dependent increase in total urine volume when compared to control. A highly significant ($p<0.001$) diuretic effect was observed at doses from 30to 50 mg/kg. The maximum increase in urine output was observed at 100 mg/kg. Diuretic effect of the extract at 100 mg/kg was comparable to that of Furosemide (Table I).The extract also demonstrated a significant increase in sodium excretion in comparison to control group. An increase ($p<0.05$) in urinary excretion of potassium was observed only at doses of 50 and 100 mg/kg (Table II).

Phytochemical analysis indicated that the extract contained certain pharmacologically active compounds such as alkaloids, saponins, flavonoids, reducing sugars, tannins and cardiac glycosides. Steroids and terpenoids were absent.

Table I: Diuretic effects of aqueous methanolic extract of *Passiflora nepalensis* in rats

| Treatment groups | Dose | Total urine volume mL/100 g/6 h | Diuresis | |
|---|---|---|---|---|
| | | | Diuretic action | Diuretic activity |
| Control | 10 ml/kg | 0.77 ± 0.12 | 1.0 | 0.28 |
| Furosemide | 10 mg/kg | 3.40 ± 0.19[a] | 4.37 | 1.0 |
| *Passiflora nepalensis* | 10 mg/kg | 1.13 ± 0.17[b] | 1.32 | 0.30 |
| *Passiflora nepalensis* | 30 mg/kg | 1.68 ± 0.20[a] | 2.16 | 0.49 |
| *Passiflora nepalensis* | 50 mg/kg | 1.81 ± 0.16[a] | 2.01 | 0.46 |
| *Passiflora nepalensis* | 100 mg/kg | 2.10 ± 0.36[a] | 2.72 | 0.62 |
| Results are expressed as mean ± SEM (n = 6); [a] $p< 0.001$ vs. control; [b] $p< 0.05$ vs. control | | | | |

Table II: Effect of *Passiflora nepalensis* on urinary electrolytes excretion in rats

| Treatment groups | Dose | Electrolytes mEq/L | | Na+/K+ |
|---|---|---|---|---|
| | | Na+ | K+ | |
| Control | 10 ml/kg | 162 ± 6.45 | 4.89 ± 0.36 | 33.1 |
| Furosemide | 10 mg/kg | 246 ± 7.90 | 7.46 ± 0.81[b] | 33.0 |
| *Passiflora nepalensis* | 10 mg/kg | 174 ± 5.48[c] | 4.94 ± 0.54 | 35.2 |
| *Passiflora nepalensis* | 30 mg/kg | 184 ±7.08[b] | 5.13 ± 0.47 | 35.9 |
| *Passiflora nepalensis* | 50 mg/kg | 191 ± 6.33[a] | 5.80 ± 0.44[c] | 33.0 |
| *Passiflora nepalensis* | 100 mg/kg | 205 ± 3.80[a] | 6.01 ± 0.51[c] | 35.0 |
| Results are expressed as means ± SEM (n = 6); [a] $p<0.001$; [b] $p<0.01$ and [c] $p<0.05$ vs. control | | | | |

**Discussion**

Medicinal plants have always been a major target for drug development. P. nepalensis, a plant native to Sikkim has been used as a diuretic to treat hypertension and other cardiovascular diseases but no pharmacological study has been carried out to test the diuretic activity of this plant. The aqueous methanolic extract of P. nepalensis was therefore evaluated for a detailed diuretic study. It is noteworthy that the extract produced a significant dose dependent diuretic effect in comparison to control. Moreover, the extract also exhibited an increase in sodium and potassium excretion when compared to control. The active principles responsible for the diuretic activity of the extract have not been elucidated but the preliminary phytochemical analysis revealed that the extract contained compounds such as flavonoids, saponins, cardiac glycosides and tannins. Diuretic activity of the extract can be linked to a number of possible mechanisms. Experimental studies have demonstrated that cardiac glycosides and tannins are endowed with both diuretic and vasodilator actions (Herrera et al., 2008). It has also been reported that saponins in association with compounds such as steroids, vitamin-D and cardiac glycosides produced diuretic response. Similarly, the flavonoids also appeared to exhibit diuretic, anti-inflammatory, and vasoprotective effects by the inhibition of arachidonic acid metabolism (Mythreyi et al., 2008). Therefore, it may be suggested that diuretic effects of the aqueous methanolic extract of P. nepalensis might be due to the presence of these substances which may act individually or in combination. Previously it has been well established that increased regional blood flow, vasodilation or an inhibition of tubular secretion contributes to an increased urinary excretion (Stanic and Samarzija, 1993; Pantoja et al., 1993). Hence, any of these processes could be associated with diuretic effect of the extract. Furthermore, an increase in sodium and potassium excretion by the extract might also be involved in diuresis. It is concluded from this study that the aqueous methanolic extract of P. nepalensis contain certain active compounds that may be responsible for the diuretic activity. Therefore, further studies are required to isolate these pharmacologically active principles and to determine their exact mechanism of action.

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