Diuretic activity of NR-ANX-C (a polyherbal formulation) in normal rats

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ABSTRACT

The present study was undertaken to investigate the diuretic properties of NR-ANX-C, a polyherbal formulation in normal rats. The study was conducted in saline primed wistar albino rats (n=6) using hydrochlorothiazide (10 mg/kg) as the reference diuretic drug. The test drug was evaluated at three different oral doses, 25mg/kg, 50mg/kg and 100mg/kg respectively. Electrolytes (Sodium, Potassium and Chloride) excretion was estimated and the diuretic activity was calculated. Results revealed that the formulation showed significant diuretic activity as evidenced by increase in the urinary output and significant increase in sodium, potassium and chloride ion excretion in the 100mg/kg test group as compared to control group. This study supports the use of NR-ANX-C, a polyherbal formulation as a diuretic agent.

Key words: Diuretic activity, NR-ANX-C, polyherbal formulation

INTRODUCTION

Man has been using herbs and plant products for its medicinal use since times immemorial. However, it is imperative that the traditional systems should be scientifically supported for their efficacy and safety.

Diuretics, either alone or in combination with other drugs, are valuable in the treatment of hypertension, congestive heart failure, ascites & pulmonary edema.\(^1\)\(^2\)\(^3\)\(^4\)\(^5\) Two widely used diuretics, thiazides and the high ceiling loop diuretic, furosemide, have been associated with a number of adverse effects, such as, electrolyte imbalance, metabolic alterations, development of new-onset diabetes, activation of the renin-angiotensin-neuroendocrine systems and impairment of sexual function.\(^1\)\(^4\) Many indigenous drugs have been claimed to have diuretic effect in Ayurvedic system of medicine but lack scientific authentication. The polyherbal preparation NR-ANX-C (a test drug supplied by Natural Remedies Pvt. Ltd., Bangalore) contains the extracts of Withania somnifera (Ashwagandha), Ocimum sanctum (Tulsi), Camellia sinensis (green tea), triphala and shilajit.

The medicinal properties of Withania somnifera, also known as ashwagandha, Indian ginseng, and winter cherry has been extensively reviewed in literature.\(^6\) The diuretic properties of this plant has been assessed in human objects.\(^7\) Ocimum sanctum, known as ‘Tulsi’ in Hindi and ‘Holy Basil’ in English is a rich source of medicinal properties.\(^8\) Leaves and seeds of Tulsi plants have been reported to reduce blood and urinary uric acid level in albino rabbits and possess diuretic property.\(^9\) Camellia sinensis (Green tea) is traditional Chinese and Indian medicine has been used as a CNS stimulant, diuretic, astringent and to improve heart health.\(^10\) Green tea contains “Theine” its chief alkaloid identical to caffeine- a polyphenol which diminishes the waste of the body i.e carbonic acid, urea, uric acid & water. Green tea consumption results in highest intake of polyphenols (caffeine) and greatest fecal and urinary excretion, compared to black tea and decaffeinated green tea.\(^11\) A recent study has concluded that C. sinensis possesses safe, mild to moderate diuretic activity.\(^12\)

Triphala is a commonly used Indian Ayurvedic herbal formulation, consisting equal parts of three medicinal dried plant fruits Emblica officinalis, Terminalia belerica and Terminalia chebula. It is an important medicine of the “Rasayana” group of Ayurveda and is believed to promote immunity, health and longevity.\(^13\) Though individual herbs in the preparation have been claimed to be excellent diuretics,\(^14\) no validation is yet sought in literature. Karpura Shilajit Bhasma at various dose levels induces significant increase in urine volume. Natriuresis was significantly increased at diuretic doses without producing significant kaluresis.\(^15\)

The presence of diuretic activity in the individual herbs may not be proved to be so in the final formulations as suggested in a similar study conducted before.\(^16\) Hence the present study was conducted to evaluate the diuretic potential of NR-NX-C, a polyherbal preparation.

MATERIALS AND METHODS

Experimental animal:

Adult male Wistar albino rats (150-200 g) from our breeding
stock were used for the study. They were housed in clean and transparent poly propylene cages with three animals in each cage and maintained at 27°C with 12: 12 h light-dark cycle for a period of 7 days prior to the study. They were fed standard rat chow and water ad libitum. The experimental procedures described were approved by the Institutional Animal Ethics Committee.

Drugs:

Hydrochlorothiazide (Micro cardicare Co.) was used as a reference diuretic drug.

Test drug: The composition of test drug NR-ANX-C (supplied by Natural Remedies Pvt. Ltd, Bangalore), a polyherbal formulation, was as follows: Withania somnifera 17 per cent (water extract of root), Ocimum sanctum 17 per cent (70% alcohol extract of leaves), Camellia sinensis 33 per cent (70% alcohol extract of leaves), triphala 25 per cent (water extract) and shilajit 8 per cent (water extract).

Preliminary Phytochemical screening:

The test drug, NR-ANX-C formulation was subjected to preliminary qualitative phytochemical investigations and was screened for the presence of secondary metabolites such as steroids, alkaloids, flavonoids and tannins using standard methods.

Acute toxicity study:

The test was carried out as suggested by Ganapaty et al. (17) Wistar albino rats, of either sex, weighing 150-200 g were divided into different groups comprising six animals each. The control group received normal saline (10 ml/kg, p.o.). The other groups received 100, 200, 500, 1000, and 2000 mg/kg of the test extract respectively, as well as, extract fractions up to 1000 mg/kg, in a similar manner. Immediately after dosing, the animals were observed continuously for the first 4 hours for any behavioral changes. Thereafter, they were kept under observation up to 14 days after drug administration to find out mortality, if any.

Evaluation of diuretic activity:

Each animal was placed in an individual metabolic cage 24h prior to commencement of the study for adaptation. The method of Lipschitz et al. (18,19) was employed for the assessment of diuretic activity. According to this method, the animals, deprived of food and water for 18 hours prior to the experiment, were divided into 5 groups (n=6). Group I animals received normal saline (25 ml/kg, p.o.); Group II received the standard diuretic Hydrochlorothiazide (10 mg/kg body weight) and Groups III - V received the test compound NR-ANX-C (25mg, 50mg and 100mg/kg body weight) respectively. Before treatment, all animals received physiological saline (0.9% NaCl) at an oral dose of 5ml/100g body weight to impose a uniform water and salt load. (20) All the drugs were freshly prepared prior to administration.

Immediately after administration, the animals were placed in metabolic cages (2 per cage), specially designed to separate urine and faeces, kept at 20°C±0.5°C. At the end of 5 hr and 24 hr, the volume of urine collected was measured. During this period, no food and water was made available to animals. The parameters noted were body weight before and after test period, total urine volume, and concentration of Na⁺, K⁺ and Cl⁻ in the urine. Na⁺, K⁺, Cl⁻ concentrations were determined by Ion Sensitive Electrode; Roche Hitachi 917 automatic analyzer.

The diuretic action of test drug was calculated by using the following formula:

\[
\text{Diuretic action} = \frac{\text{Urinary excretion of test drug group}}{\text{Urinary excretion in control group}}
\]

Statistical Analysis:

The results were expressed as mean ± SEM. The data was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test. A value of P < 0.05 was considered as statistically significant.

RESULTS:

The preliminary phytochemical analysis revealed the presence of withanolides, ursolic and ethanolic acids, polyphenols, fulvic and humic acid and tannins. (Table no. 1)

Acute toxicity tests:

The test extract showed no signs of acute toxicity as evidenced by the absence of mortality or visible adverse effects in the animals during the study period. No macroscopic alterations were noted in the viscera of the treated rats.

Effect on urine volume:

There was no evidence of dehydration and the animals were found normal at the observed 5hr and 24hr intervals. The reference diuretic hydrochlorothiazide, significantly increased the urine output when compared to control (P < 0.01), the diuretic index being 2.66. The test drug at 25 and 50 mg/kg doses, showed a small increase in the urine volume although, it was statistically insignificant. As the dose was increased to 100mg/kg, the extract however, showed significant diuresis as showed in table no 2; (P < 0.01). The diuretic index of the test drug at 100mg/kg (1.94) was less than that of hydrochlo-

Table 2: Effect of oral administration of the NR-ANX-C formulation on urinary volume excretion:

<table>
<thead>
<tr>
<th>Group</th>
<th>Urine volume (ml/100g/5hr)</th>
<th>Urine volume (ml/100g/24 hrs)</th>
<th>Diuretic index (5 hr interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.68 ± 0.16</td>
<td>2.98 ± 0.14</td>
<td>2.66</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>4.47 ± 0.3*</td>
<td>5.30 ± 0.37*</td>
<td>2.56</td>
</tr>
<tr>
<td>NRANXC extract (25mg/kg)</td>
<td>1.72 ± 0.15</td>
<td>2.68 ± 0.43</td>
<td>1.05</td>
</tr>
<tr>
<td>NRANXC extract (50mg/kg)</td>
<td>2.79 ± 0.46</td>
<td>3.83 ± 0.18</td>
<td>1.66</td>
</tr>
<tr>
<td>NRANXC extract (100mg/kg)</td>
<td>3.27 ± 0.4*</td>
<td>4.36 ± 0.56</td>
<td>1.94</td>
</tr>
</tbody>
</table>

Values are expressed in means±SEM; *P <0.01 compared with control group (ANOVA followed by Dunnett’s test). Diuretic index = volume of test group/volume of control group.

Table 3: Effect of oral administration of the NR-ANX-C formulation on urinary electrolyte excretion

<table>
<thead>
<tr>
<th>Groups</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Cl⁻</th>
<th>Saluretic index Na/K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>152±2.1</td>
<td>71.1±2.17</td>
<td>233±3.62</td>
<td>1.05</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>1792±7.9*</td>
<td>106.5±3.7*</td>
<td>430±4.8*</td>
<td>1.18</td>
</tr>
<tr>
<td>NRANXC extract (25mg/kg)</td>
<td>219±3.23*</td>
<td>120±2.77*</td>
<td>454±5.7*</td>
<td>1.44</td>
</tr>
<tr>
<td>NRANXC extract (50mg/kg)</td>
<td>225±4.1*</td>
<td>105±3.87*</td>
<td>484±5.3*</td>
<td>1.48</td>
</tr>
<tr>
<td>NRANXC extract (100mg/kg)</td>
<td>357±3.98*</td>
<td>126±2.6*</td>
<td>676±4.66*</td>
<td>2.35</td>
</tr>
</tbody>
</table>

Values are expressed in means±SEM; *P <0.01 compared with control group (ANOVA followed by Dunnett’s test). Saluretic index = volume of test group/volume of control group.

Effect on urinary electrolyte excretion:

As indicated in table no 3, the test drug, when compared to the control group, showed a significant increase in the excretion of sodium, potassium and chloride excretion in dose dependent manner. These changes were also reflected in a dose dependent increase in the Na/K ratio. The increase in the electrolyte excretions was more than that of the standard drug, hydrochlorothiazide at all the doses used indicating a stronger saluretic effect than hydrochlorothiazide.

DISCUSSION:

In the present study, the diuretic effect of orally administered polyherbal formulation, NR-ANX-C was evaluated in normal rats. The pharmacological response was compared with that produced by hydrochlorothiazide, a widely used thiazide diuretic in clinical practice.

Diuresis has two components: increase in urine (water secretion) and a net loss of solutes (i.e. electrolytes) in the urine. These processes result from suppression of renal tubular reabsorption of water and electrolytes into the blood stream. The reference drug, hydrochlorothiazide inhibits the Na⁺/Cl⁻ symporter (co-transporter system) in the distal convoluted tubule by competing for the Cl⁻ binding site and increasing the excretion of Na⁺ and Cl⁻. It has been reported that hydrochlorothiazide increases the urinary excretion of both Na and K by 50-60% over controls after a single oral dose in normal rats. In the present study, hydrochlorothiazide showed a marked excretion of both water and ions, typical of saluretic diuretics of thiazide type. Results from the NR-ANX-C formulation, showed a significant increase in urine output after 5 hrs of administration at a dose of 100mg/kg in saline primed rats. However, it significantly increased the electrolyte excretion at all the doses employed. (P < 0.01) The diuretic activity of NR-ANX-C was dose dependent indicating that this effect is genuine, intrinsic, and causal; possibly receptor mediated. At higher doses, the increase in electrolyte excretion was higher compared to the standard drug, hydrochlorothiazide indicating that the formulation has a stronger saluretic action than hydrochlorothiazide. The increase in the ratio of concentration of excreted sodium and potassium ions indicates that the extract increases sodium ion excretion to a greater extent than potassium, which is very essential quality of a good diuretic with lesser hyperkalemic side effects.

Presence of phytoconstituents like withanolides- withaferin A and witharistatin, (steroidal lactones), ursoic acid & oleic acid (pentacyclic triterpinoids), and caffeine (methylxanthine) have been previously found to be responsible for diuretic activities in plants. The presence of the above constituents in NR-ANX-C formulation may be responsible for the observed diuretic activity. One mechanism by which NR-ANX-C formulation can elevate urinary Na⁺ level is by inhibiting the Na⁺ resorption in the nephron. This mode of action is likely to be operative here as caffeine which is reported to be present in the test drug is known to inhibit Na⁺ resorption at nephrons. 

It is very essential to isolate the active principles and evaluate the individual components of the formulation for their individual as well as synergistic effects.

CONCLUSION:

The present study validates the use of the polyherbal formulation NR-ANX-C as a diuretic agent. Diuretics are clinically used in patients with salt and water overload due to host of conditions such as pulmonary edema, heart failure, ascites, hypertension etc. NR-ANX-C formulation can be used as non toxic natural therapeutic agent in the treatment of such conditions. Further studies regarding the exact mechanism of action and synergistic effects of the individual components are warranted.

REFERENCES:


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