Research Article

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EVALUATION OF ACUTE TOXICITY STUDY AND DIURETIC ACTIVITY OF URAL SYRUP

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ABSTRACT
There are considerable amount of scientific evidences available for diuretic activity of individual ingredients of a polyherbal formulation Ural Syrup however no evidence has been found which proves overall safety and efficacy of the formulation. The present study was undertaken to investigate safety as well as Diuretic activity of this polyherbal formulation- Ural Syrup. Acute toxicity study was carried out as per OECD Guideline 420. The Ural syrup (1.8mL/kg and 3.6mL/kg) was tested for its diuretic activity on rat model. Hydrochlorothiazide (HCTZ) (10mg/kg) was used as positive control in study. The diuretic effect of the Ural syrup was evaluated by measuring urine volume, pH and excretion of sodium and potassium content. In result, significant increase in urine volume was observed at both dosage levels of Ural syrup in comparison to normal control group. There was no significant change observed in pH of urine. The excretion of Sodium was also found significantly increased at both dosage levels but more in TEDx2 group with respect to TED group. Potassium excretion was found increased only at TEDx2 group as compared to normal control group. It can be concluded that Ural syrup produced considerable diuretic effect which appeared to be comparable to that produced by the reference diuretic HCTZ. There was no lethality and toxic reaction found among the tested animals. The present study provides scientific support that Ural syrup can be employed as a safe and effective diuretic drug.
Keywords: Ural syrup, acute toxicity study, Diuretic, Hydrochlorothiazide.

INTRODUCTION

The diuretics are drugs that act on kidney and are able to increase the volume of urine excreted. Drug-induced diuresis is beneficial in many life threatening disease conditions such as cardiac failure, chronic and moderate cardiac insufficiencies, acute edema of the lung, nephritic edema syndrome, arterial hypertension, diseases related with the retention of fluids etc[1,2]. Ural syrup is a patent and proprietary Ayurvedic formulation which contains extract of *Tribulus terrestris* (Gokshur)[3], *Berginia ligulata* (Pashanbhed)[4], *Boerhaavia diffusa* (Punarnava)[5], *Crataeva nurvala* (Varun)[6], *Raphanus sativus* (Mulika)[7], *Dolichos biflorus* (Kulathi)[8], *Smilax china* (Chopchini)[9] and powder of Sodii carbonas (Swarjikakshar)[10], Borax (Shuddha Tankan)[11], Potasii carbonas (Yavakshar)[12], Kala namak (Black Salt)[13] and *Citrus acida* (Nimbuk)[14]. It is manufactured and marketed by Vasu Healthcare Pvt. Ltd., Vadodara, Gujarat, India. Majority of ingredients of Ural syrup are well reported in Ayurvedic texts and scientific research publications for variety of activities like diuretic, alkalizing, anti-inflammatory etc. However, no such evidence was found available which proves safety and efficacy of their combinations. Hence, the present study was undertaken to investigate overall safety along with diuretic activity of such polyherbal combination Ural syrup.

MATERIALS AND METHODS

Test Drug and Dosage

Test drug (Ural syrup) was used for evaluation of acute toxicity study and diuretic activity. For acute toxicity study 2000mg/kg and 5000mg/kg single dose was administered orally. For diuretic activity, dose of the test drug was fixed by extrapolating the human dose to laboratory animals based on body surface area ratio as per the table of Paget and Barnes. Test drug was administered once in a day at two different dose levels i.e. 1.8mL/kg (p.o) consider as Therapeutic Effective Dose (TED) and 3.6mL/kg (p.o) consider as double of Therapeutic Effective Dose (TEDx2).

Experimental Animals

Healthy Swiss albino mice (20-25g) of either sex were taken for acute toxicity study and Wistar albino rats (200-250g) were taken to assess diuretic activity. Both were procured from S.K.Patel College of Pharmaceutical Education And Research, Ganpat University, Kherva, Mehsana, Gujarat, India. All the experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) and with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) IAEC/SKPCPER/2010-02/18 and 19 respectively for acute toxicity study and diuretic activity, Ministry of Social Justice and Empowerment, Government of India. Animals were housed in polypropylene cages, maintained under standardized condition (12h light/dark cycle, 24°C, 35 to 60% humidity) and provided free access to standard pellets diet and purified drinking water ad *libitum.* The animals were deprived of food for 24h before experimentation but allowed free access to water throughout.

Acute Oral Toxicity Study

For acute toxicity study on mice, 'Fixed dose' method of the Organization for Economic Cooperation and Development (OECD) guideline 420 was followed.[15,16] The formulation was administered by gavages (orally) at single doses of 2000mg/kg and 5000mg/kg. The animals

had free access to water and food throughout the experiment, except for the fasting period before the oral administration of the single dose of the Formulation. The general behaviour of rats was continuously monitored for 2h. After period of 24 h, 72 h, 7days and 14days they were observed for changed in body weight and lethality or death.

Evaluation of Diuretic activity

Grouping of animals

Rats were randomly divided into four groups with six animals in each group. Group I: Normal Control, Group II: Hydrochlorothiazide- reference standard (10mg/kg, p.o), Group III: Ural Syrup (TED) (1.8mL/kg, p.o), Group IV: Ural Syrup (TED x 2) (3.6mL/kg, p.o)

Experimental Protocol

Diuretic activity was determined by following the method of Kau ST *et al.* (1984) with minor modifications.[17] Rats were randomly divided into four groups with six animals in each group as follows

- Group I: Normal Control (5mL/kg de-ionized water, p.o.)
- Group II: Hydrochlorothiazide- reference standard (10mg/kg, p.o.),
- Group III: Ural Syrup (TED) (1.8mL/kg, p.o.),
- Group IV: Ural Syrup (TED x 2) (3.6mL/kg, p.o.)

In all cases, the volume of the dose was administered 5mL/kg body weight. The animals were fasted overnight (18h) prior to the test but with free access to tap water only and then were given an oral loading of normal saline (0.9%) of 0.05mL per g body weight. Immediately after administration, rats were paired and placed in metabolism cages. Urine was collected in a graduated cylinder and its volume was recorded at intervals of every 1h for 5h. Cumulative urine excretion was calculated in relation to body weight and expressed as mL/100 g b.w./5h. Electrolytes (Na+ and K+) concentrations and pH were estimated from the urine sample of each pair of rats at the end of the experimental period (5h).

Measurement of Urine Output and Analysis of electrolytes

Na+ and K+ concentrations were measured using a Toshniwal group model TCM-35 flame photometer. The instrument was calibrated with standard solutions containing different concentrations of Na+ and K+. Fresh urine sample was measured for pH with a pH meter (Lab India).

Statistical analysis

The results were expressed as mean values ± S.E.M. (standard error of mean) of six pairs of rats. Statistical comparison was carried out by analysis of variance (ANOVA). The statistical analysis was carried out with software, Graph pad Prism®, version 5.0. The results were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

In the acute oral toxicity study, the animals in the test did not manifest any signs of toxicity or deaths at both dose level 2000mg/kg and 5000mg/kg. The body weights of all the mice were increased after the oral administration of Ural Syrup and the marked % gain were observed on 7thday and 14thday. The results of change in body weight are shown in Table 1 and Table 2. The results of the evaluation carried out on Ural Syrup are listed in Table 3 and Table 4. Data comparison for Urine Volume, Sodium excretion and Potassium excretion were also mentioned in the plotted graphs in Figure 1, Figure 2 and Figure 3 respectively.

Table 1: Effect of Ural Syrup on the body weight of mice at 2,000mg/kg body weight

Product	Dose	Mean body weight (g)				
		0 day	7th day	% Gain on 7th day	14th day	% Gain on 14th day
Ural Syrup	2000mg/kg	23.67±0.88	30.33 ± 0.88	28.39 ± 4.49	36.33 ± 0.33	54.00 ± 7.21

Table 2: Effect of Ural Syrup on the body weight of mice at 5,000mg/kg body weight

Product	Dose	Mean body weight (g)				
		0 day	7th day	% Gain on 7th day	14th day	% Gain on 14th day
Ural Syrup	5000mg/kg	38.93±1.18	41.5±0.58	6.60±2.43	43.00±0.58	10.45±3.13

Table 3: Effect of Ural Syrup and HCTZ on urine volume, diuretic index and pH

Treatment	Dose (mg/kg p.o.)	Urine Volume (mL/100g/5h)	Diuretic Index	pH
Normal Control	---	3.33±0.032	---	7.49±0.053
HCTZ	10mg/kg	5.55±0.082***	1.66	7.42±0.036
Ural Syrup (TED)	1.8mL/kg	4.77±0.066***	1.43	7.38±0.036
Ural Syrup (TEDx2)	3.6mL/kg	5.15±0.030***	1.54	7.36±0.036

Data represent in Mean ± SEM. Where n=6, $^{***}p < 0.001$ compared with the control group.,
Diuretic index = volume treated group/ volume control group.

Table 4: Effect of Ural Syrup and HCTZ on sodium and potassium excretion in urine

Treatment	Dose (mg/kg p.o.)	Sodium (meq/100g/5h)	Potassium (meq/100g/5h)
Normal Control	---	4.72±0.056	11.53±0.056
HCTZ	10mg/kg	20.69±0.094***	29.68±0.047***
Ural Syrup (TED)	1.8mL/kg	13.29±0.096**	12.75±0.056
Ural Syrup (TEDx2)	3.6mL/kg	19.24±0.052***	21.94±0.054**

Data represent in Mean ± SEM. Where n=6, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with the control group.

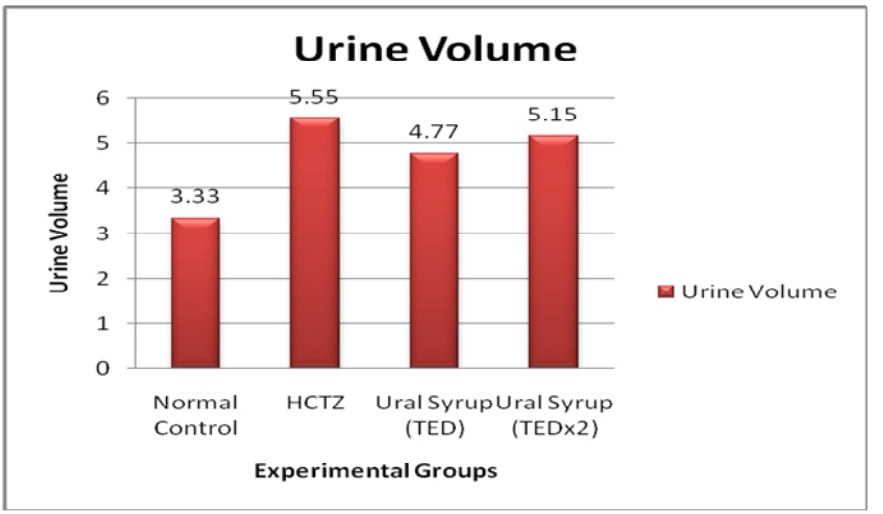

Figure 1: Effect of Ural syrup on urine volume

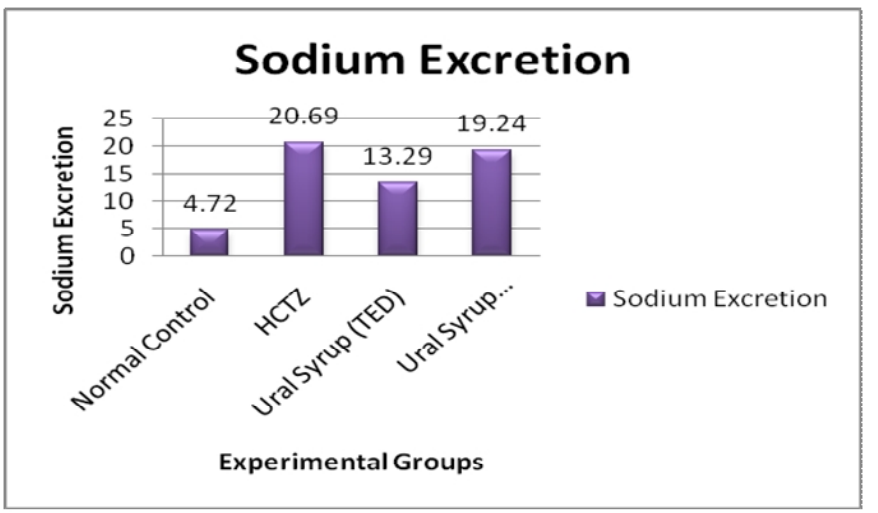

Figure 2: Effect of Ural syrup on sodium excretion

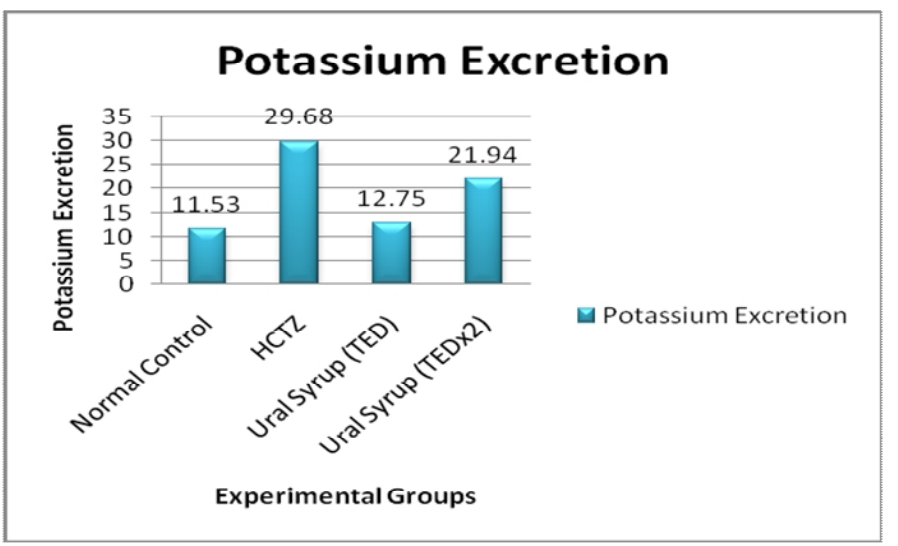

Figure 3: Effect of Ural syrup on potassium excretion

Urine volume and pH

As per Table 3 and Figure 1 the reference diuretic HCTZ increased urine volume to 5.55±0.082 While Ural syrup at the dose of 1.8mL/kg body weight and 3.6mL/kg body weight showed marked diuresis during 5h i.e. 4.77±0.066 ($P < 0.001$) and 5.15±0.030 ($P < 0.001$) respectively as compared to the control group. There was no significant difference observed in pH of urine (Table 3). Diuretic index shows that diuretic action of both the test group is comparable to reference standard especially Ural Syrup (TEDx2) group (Table 3).

Electrolyte excretion

Table 4 and Figures 2 and 3 shows the excretion level of urinary electrolyte content following the administration of the Ural syrup. The dose of 1.8mL/kg Ural Syrup produced a significant increase in Na^+ excretion ($P< 0.01$), compared with the control group. No significant excretion of K^+ was observed. The dose of 3.6mL/kg Ural Syrup also produced a significant increase in the Na^+ excretion ($P < 0.001$) and K^+ excretion ($P<0.01$). However, only measure of the ionic content of the urine was observed and found to be increased in a dose-dependent manner in Ural syrup treated groups. Based on above results, the diuretic effect of Ural syrup was confirmed by increase in excretion of sodium and potassium and urine volume. Observed diuretic effect of Ural Syrup is thought to be due to stimulation of regional blood flow or initial vasodilatation or by producing inhibition of tubular re-absorption of water and anions.

CONCLUSION

The results obtained in oral acute toxicity and diuretic study provide a scientific basis to explain the use of Ural Syrup as a safe diuretic formulation. Double dose level of Ural syrup was more effective with respect to single dose level.

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