# Evaluation of Diuretic Activity of Isatin Derivatives in Wistar Rats

E. Venkateshwarlu[a,b,*], B.S. Sharvana Bhava[b], K. Umasankar[b] and J. Venkateshwar Rao[c]

[a]*Department of Biotechnology, Acharya Nagarjuna University, Guntur, 522510, India*

[b]*Vaagdevi College of Pharmacy, Hanamkonda,Warangal, 506001 , India*

[c]*TallaPadmavathi College of Pharmacy, Orus, Warangal, 506002, India*

**Abstract:** In the present studies we have synthesized ten derivatives of novel isatin-3-[$N^2$-(2-benzalaminothiazol-4-yl)]hydrazone and characterized by the help of physical and spectral data. The selected male Wistar albino rats were divided into different groups: control, urea (1 g/kg), furosemide (5 mg/kg), of isatin derivatives (**Ia-Ij**, 10 and 100 mg/kg) treated groups. All the drugs were administered orally and animals were pretreated with normal saline (25 ml/kg) before start the experiment. The urine volume (in ml) and content of $Na^+$, $K^+$ & $^-Cl$ were measured in the urine of rats at 5$^{th}$ hour. The volume of urine output increased significantly ($p<0.05$) in urea, furosemide and also isatin derivatives treated groups when compared with control groups. The diuretic activity of isatin derivatives were exhibited moderated to less when compared with furosemide. The diuretic response with its electrolyte of the isatin derivatives was highly remarkable in comparison with control group. Based on the results the isatin derivatives show hypernatremic, hypercholermic and hyperkalemic diuretics as conventional diuretics (furosemide).

**Keywords:** Isatin hydrazones, diuretic activity, hypernatremic, hypercholermic and hyperkalemic.

## 1. INTRODUCTION

Diuretics are the substances causing an increased production of urine in an organism thus decreasing the fluid volume in its tissues and 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. Most diuretics increase urine volume by inhibiting the reabsorption of sodium and chloride ions in the renal tubule; they also modify renal handling of potassium, calcium, magnesium and aureate. Drug-induced diuresis is beneficial in many life threatening disease conditions such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure, hypertension, and pregnancy toxaemi [1]. Schappert reported more than 45 million peoples treated by diuretic alone in cardiac patients [2]. Diuretics can also be helpful in diluting the ion contents of the urine, leading to reduction in the super saturation of stone forming ions and also help in expulsion of crystals, thus preventing recurrent renal stones [3]. Two widely used diuretics, namely hydrochlorothiazide and furosemide have been associated with a number of adverse effects, such as, electrolyte imbalance, metabolic alterations, development of new-onset diabetes, activation of the rennin–angiotensin–neuroendocrine systems and impairment of sexual function [4, 5]. Recently developed new genetic target for diuretic therapy in patients with fluid overload like those with congestive heart failure, liver cirrhosis or kidney failure were identified by university of Cincinnati researchers and this helped for patients who have resistance to diuretic therapy and it lead to first new diuretic therapy in 25 years [6]. Diuretic use is associated with better learning and memory in older adults in the Ginkgo evaluation of memory study. In this study potassium-sparing diuretic use was associated with better verbal learning and memory measured by California Verbal Learning Test as compared with no antihypertensive medication users and other antihypertensive medication users [7].

Isatin and its derivatives are widely used for the synthesis of different heterocyclic compounds, amongst which a great number of drug candidates have been found. The variety of applications of isatin derivatives is due to their extremely wide range of biological activities. Isatin is an endogenous indole present in mammalian tissues and fluids [8] and it is also distributed in rat brain and other tissues the highest concentrations in the brain are found in the hippocampus and cerebellum [9]. Isatin derivative had wide range of biological activities like antimicrobial [10], anti-tuberculosis [11], anthelmimtic [12], antioxidant, antiatherogenic [13], anti-proliferative [14], anti-tumor [15], antiepileptic [16], anti-inflammatory, analgesic, antipyretic [17] and diuretic activity [18] respectively. From the above literature, we synthesized novel derivatives of isatin and were screened for their diuretic activity on Wistar albino rats.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and Drugs

Carboxy methyl cellulose (CMC) was purchased from S.D. Fine, Mumbai, India. Urea procured from

*Address correspondence to this author at the Department of Biotechnology, Acharya Nagarjuna University, Guntur, 522510, India; Tel: +919848835092; Fax: +918702460108; E-mail: eggadivenkey@gmail.com

ISSN: 2223-3806 / E-ISSN: 1927-5951/13 

Sisco Research laboratories Pvt Ltd., Mumbai, India. Furosemide was obtained from Sigma-Aldrich chemicals Pvt Ltd., Mumbai, India.

### 2.2. Synthesis of Isatin Derivatives

#### *2.2.1. General Procedure for Synthesis of Isatin Hydrazones [19]*

#### *2.2.2. General Procedure for Synthesis of Isatin-3-[$N^2$-(chloroacetyl)]hydrazones [20]*

#### *2.2.3. General Procedure for Synthesis of Isatin-3-[$N^2$-(2-aminothiazol-4-yl)]hydrazones [21]*

#### *2.2.4. Synthesis of Isatin-3-[$N^2$-(2-benzalamino-thiazol-4-yl)]hydrzones*

A solution of an appropriate isatin-3-[$N^2$-(2-aminothiazol-4-yl)]hydrazone (0.01 mol) in ethanol (60 ml) added 2-3 drops of glacial acetic acid and various aromatic aldehyes (0.01 mol), the reaction mixture were heated at refluxed for about 8-10 hr. Completion of the reaction was monitored by TLC. Solvent present in the reaction mixture was evaporated under vacuum and the solid was collected and washed with cold petroleum ether, further purified by recrystallization from suitable solvent.

### 2.3. Experimental Animals

In this study Male Wistar albino rats (150-180 gm) were selected and procured from Mahaveer enterprises, Hyderabad, India. All animals were maintained under environmentally controlled conditions of 25±2 °C, relative humidity of 45 to 55 % and 12 hr light-12 hr dark cycle. The animals were acclimatized to laboratory conditions at least one week before starting the experiment and they had free access to food and water *ad labitum*. The study protocol was approved by Institutional Animal Ethics Committee (IAEC NO: 1047/ac/07/CPCSEA).

### 2.4. Experimental Procedure

Selected animals were divided into different groups (n=6). All the animals received normal saline (25 ml/kg, b.w) orally, before starting the experiment. The first group was control group and received 0.1 % sodium CMC, second group received Urea (1 g/kg), third group received standard (furosemide, 5 mg/kg) and remaining groups were treated with test compounds (**Ia-Ij,** 10 and 100 mg/kg*, p.o*). Immediately after administration of the drugs, rats were individually placed in metabolic cages with total withdrawal of food and water *ad libitum.* The volume of urine is collected from individual animal at $5^{th}$ hr [22, 23]. The urine volume (ml) was measured and assayed for $Na^+$, $K^+$ and $^-Cl$ concentrations [24-26]. The $Na^+$ and $K^+$ were measured by a flame photometric method (Chemito 1020) while $^-Cl$ concentration was determined by titration with silver nitrate solution (N/50) using 3 drops of 5% potassium chromate solution as an indicator [27]. The instrument was calibrated with standard solutions containing different concentrations of sodium and potassium [28].

### 2.5. Measurement of Urinary Excretion, Diuretic Activity and Diuretic Action [29, 30]

The urinary excretion, diuretic activity and diuretic action of the control, urea, furosemide and isatin derivatives (**Ia-Ij**) treated groups were calculated from the following equations:

Urinary Excretion = Total Urinary output (Vo)/Total liquid administrered (V1) X 100

Diuretic Action = U.E.in test group (UEt)/U.E.in control group (UEc)

Diuretic Activity = Diuretic action of drug (DAt)/Diuretic action of urea (DAu)

### 2.6. Evaluation of Natriuretic, Saluretic and Carbonic Anhydrase Inhibition

For natriuretic activity the ratio of $Na^+/K^+$ was calculated. The sum of $Na^+$ and $^-Cl$ excretion was calculated as a parameter of saluretic activity. For estimation of carbonic anhydrase enzyme inhibition the ratio of $^-Cl/Na^+ + K^+$ was calculated [31].

### 2.7. Evaluation of Diuretic Index and Electrolytic Excretion Index

The diuretic and electrolyte excretion index of the all treated groups were calculated from test group and control group [32].

### 2.8. Statistical Analysis

Results were expressed as Mean ± SD, statistical significance was calculated by applying one way ANOVA. $P<0.05$ was considered as significant (Newman-Keuls multiple comparison test).

## 3. RESULTS

### 3.1. Synthesis of Isatin-3-[$N^2$(2-benzalaminothiazol-4-yl)]hydrazones

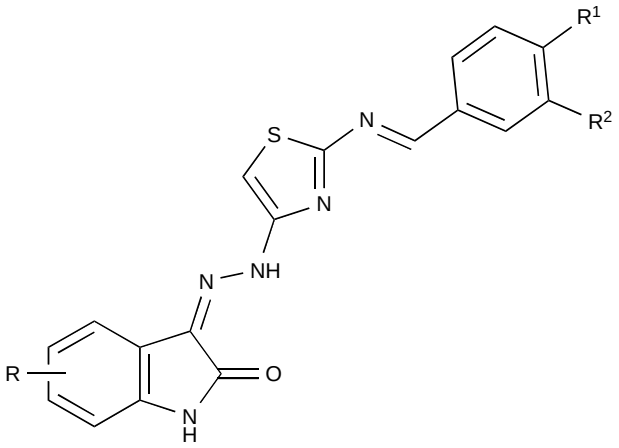
The isatin-3-[$N^2$-(2-benzalaminothiazol-4-yl)] hydra-zones have been synthesized by the following

sequence of chemical reactions. The respective isatins were reacts with 99% hydrazine hydrate offered the isatin hydrazones. The isatin-3-[$N^2$-(chloroacetyl)] hydrazones were prepared by a reaction of respective isatin hydrazones with chloroacetyl chloride. Condensation of chloroacetyl derivatives of isatin hydrazones with thiourea in absolute ethanol given isatin-3-[$N^2$-(2-aminothiazol-4-yl)]hydrazone. Finally the title compounds prepared by respective isatin aminothiazolyl hydrazone were condensed with different aromatic aldehydes. However, intermediates and final compounds have been further purified by recrystallization from appropriate solvents(s) and characterized by their physical and spectral data. The representative compound in the series **Ia** [isatin-3-[$N^2$-(2-benzalaminothiazol-4-yl)]hydrazones] was characterized by their spectral data. $^1$H NMR spectrum (DMSO-$d_6$, δ, ppm) at 10.94 (s, 1H, lactam), 8.67 (s, 1H, NNH), 7.91 – 8.07 (m, 9H, Ar-H), 7.47 (s, 1H, N=CH), 6.97 (s, 1H, thiazole-H). Mass spectrum, m/z: 347.7 (7%), 272.7 (100%) and 244.7 (25%). The details of isatin derivatives were given in Table **1**.

### 3.2. Effects on Urine Output and Diuretic Activity

Urea, furosemide and isatin derivatives with both doses (**Ib, Id, If, Ih** and **Ij**) increase the urine output significantly ($p<0.001$) when compared with control group. The remaining isatin derivatives (**Ie, Ii, Ia, Ic** and **Ig**) also increase the urine output, but low dose (10mg/kg) of the following derivatives like (**Ie** and **Ii**) and (**Ia, Ic** and **Ig**) shows significant urinary excretion ($p<0.01$, $p<0.05$) respectively and with high dose (100mg/kg) will show the significant effect ($p<0.001$) when compared with control group. The high dose effectively excretes more volume of urine when compared to control group. From the above results isatin derivatives **Ij** and **Ib** showed maximum diuretic activity at both doses (10 &100 mg/kg) and other derivatives **(If** and **Ih**) with 100 mg/kg show maximum diuretic activity, with 10 mg/kg show moderate activity. The diuretic activity of a drug is considered to be good if it is above 1.50, moderate if it is within 1.00-1.50, little if it is between 0.72-1.00 and nil if it is less than 0.72.In this respect few isatin derivative (**Ia, Ic** and **Ig**) showed nil diuretic activity at both dose levels and rest of the

**Table 1: Physical Data of Isatin-3-[N$^2$-(2-benzalaminothiazol-4-yl)]hydrazones**




| Compound | R | R$^1$ | R$^2$ | MF | MW |
|---|---|---|---|---|---|
| **Ia** | H | H | H | $C_{18}H_{13}N_5OS$ | 347 |
| **Ib** | H | Cl | H | $C_{18}H_{12}ClN_5OS$ | 381 |
| **Ic** | H | $N(CH_3)_2$ | H | $C_{20}H_{18}N_6OS$ | 390 |
| **Id** | H | OH | $OCH_3$ | $C_{19}H_{15}N_5O_3S$ | 393 |
| **Ie** | 5-$CH_3$ | Cl | H | $C_{19}H_{14}ClN_5OS$ | 395 |
| **If** | 5-$CH_3$ | OH | $OCH_3$ | $C_{20}H_{17}N_5O_3S$ | 407 |
| **Ig** | 5-$CH_3$ | H | H | $C_{19}H_{15}N_5OS$ | 361 |
| **Ih** | 5-Cl | OH | $OCH_3$ | $C_{19}H_{14}ClN_5O_3S$ | 427 |
| **Ii** | 5-Cl | Cl | H | $C_{18}H_{11}Cl_2N_5OS$ | 416 |
| **Ij** | 5-$NO_2$ | OH | $OCH_3$ | $C_{19}H_{14}N_6O_5S$ | 438 |

**Table 2: Dose Response Diuretic Activity of Isatin Derivatives in Normal Rats at 5th hour by Oral Administration**

| Treatment | Dose (mg/kg) | Volume of Urine (ml) | Urinary Excretion (Vo/V1) X 100 | Diuretic action $UE_t/UE_c$ | Diuretic Activity $DA_t/DA_u$ |
|---|---|---|---|---|---|
| **Control** | 25 ml of 0.9%NaCl | 0.72±0.16 | 18.41 | ----- | ---- |
| **Urea** | 1g./kg | 1.40±0.18*** | 35.00 | 1.90 | ---- |
| **Furosemide** | 5 | 2.52±0.14*** | 64.61 | 3.50 | 1.84 |
| **Ia** | 10 | 0.95±0.03* | 22.61 | 1.22 | 0.64 |
| | 100 | 1.00±0.02** | 25.00 | 1.35 | 0.71 |
| **Ib** | 10 | 1.86±0.16*** | 53.14 | 2.88 | 1.51 |
| | 100 | 2.12±0.02*** | 55.78 | 3.03 | 1.59 |
| **Ic** | 10 | 0.98±0.02* | 21.77 | 1.18 | 0.62 |
| | 100 | 1.02±0.02** | 24.28 | 1.31 | 0.69 |
| **Id** | 10 | 1.80±0.03*** | 45.00 | 2.44 | 1.28 |
| | 100 | 2.00±0.16*** | 52.63 | 2.85 | 1.50 |
| **Ie** | 10 | 1.05±0.02** | 25.00 | 1.35 | 0.71 |
| | 100 | 1.68±0.02*** | 43.07 | 2.33 | 1.23 |
| **If** | 10 | 1.80±0.03*** | 51.42 | 2.79 | 1.47 |
| | 100 | 2.04±0.15*** | 56.66 | 3.07 | 1.62 |
| **Ig** | 10 | 0.95±0.19* | 21.11 | 1.14 | 0.60 |
| | 100 | 0.99±0.02** | 23.02 | 1.25 | 0.65 |
| **Ih** | 10 | 1.82±0.02*** | 47.89 | 2.60 | 1.36 |
| | 100 | 2.06±0.18*** | 54.21 | 2.94 | 1.54 |
| **Ii** | 10 | 1.02±0.02** | 23.72 | 1.28 | 0.67 |
| | 100 | 1.59±0.04*** | 39.75 | 2.15 | 1.13 |
| **Ij** | 10 | 1.90±0.04*** | 54.28 | 2.94 | 1.55 |
| | 100 | 2.18±0.02*** | 58.91 | 3.20 | 1.68 |

Values are expressed as mean ± SD (Number of animals, n=6); $V_o$= Total urinary output; $V_1$= Total fluid input; UEt = Urinary excretion in test group; UEc = Urinary excretion in control group; DAt = Diuretic action of the test sample; DAu = Diuretic action of the Urea; *, ** and *** indicates (*) p<0.05, (**) p<0.01 and (***) p<0.001vs.control(Newman-Keuls multiple comparison test).

derivatives (**Id, Ie** and **Ii**) showed moderate diuretic activity (Table **2**).

### 3.3. Effects on Electrolyte Excretion

The diuretic responses with its electrolyte excretion potency of the isatin derivatives were highly moderate in comparison with control animals. The isatin derivatives at doses of 10 and 100 mg/kg showed increase in $Na^+$, $^-Cl$ excretion, accompanied by the excretion of K+. The 10 and 100 mg/kg doses of the following derivatives (**Ij, Ib, Ih, Id** and **If**) enhance significantly the urinary excretion of sodium (p<0.001), potassium (p<0.001), chloride (p<0.001) and few derivatives (**Ie** and **Ii**) show significant (p<0.01, p<0.001) increase in excretion of electrolytes and remaining derivatives (**Ia, Ic** and **Ig**) also show significant effect (p<0.05, p<0.01) compared with control group.

### 3.4. Effects on Natriuretic, Saluretic and Carbonic Anhydrase Inhibition (CAI)

Table **3** shows the natriuretic, saluretic and CAI activity after oral administration of isatin derivatives, urea, furosemide and control groups. All the isatin derivatives at both doses (10 and 100 mg/kg) showed marked saluretic, natriuretic and CAI activity comparable to controlgroup. The natriuretic ratio values > 2 indicate favorable natriuretic activity. With decreasing the CAI ratio values <0.8 slight to strong CAI activity could assumed [33]. From the above results it can be suggested that the isatin derivatives (**Ib, Ie, If, Ih, Ii** and **Ij**) are an effective hypernatremic,

**Table 3: Electrolytes Excretion (mMol/L), Saliuretic, Natriuretic and CAI Activity of Extract of Isatin Derivatives in Normal Rats at 5th hour by Oral Administration**

| Treatment | Dose(mg/kg) | $Na^+ + {}^-Cl$ | $Na^+ / K^+$ | ${}^-Cl / Na^+ + K^+$ |
|---|---|---|---|---|
| **Control** | 25 ml of 0.9%Nacl | 226.90±1.73 | 2.55±0.05 | 0.552±0.01 |
| **Urea** | 1g/kg | 241.65±3.08*** | 2.42±0.06*** | 0.545±0.02 |
| **Furosemide** | 5 | 357.30±3.47*** | 2.23±0.02*** | 0.480±0.02*** |
| **Ia** | 10 | 232.45±2.89* | 2.45±0.04** | 0.546±0.03 |
| | 100 | 233.40±3.18** | 2.43±0.04*** | 0.543±0.02 |
| **Ib** | 10 | 297.59±4.07*** | 2.41±0.02*** | 0.471±0.01*** |
| | 100 | 333.51±3.38*** | 2.23±0.03*** | 0.471±0.01*** |
| **Ic** | 10 | 232.80±3.10* | 2.44±0.02*** | 0.545±0.01 |
| | 100 | 233.71±2.98** | 2.42±0.03*** | 0.543±0.01 |
| **Id** | 10 | 239.40±3.15*** | 2.22±0.03*** | 0.537±0.01 |
| | 100 | 274.33±3.25*** | 2.04±0.04*** | 0.530±0.02 |
| **Ie** | 10 | 233.90±3.04** | 2.43±0.02*** | 0.542±0.03 |
| | 100 | 253.87±3.20*** | 2.44±0.03*** | 0.548±0.02 |
| **If** | 10 | 276.27±3.21*** | 2.84±0.03*** | 0.493±0.02*** |
| | 100 | 315.57±3.13*** | 2.34±0.05*** | 0.493±0.01*** |
| **Ig** | 10 | 231.93±2.95* | 2.46±0.06* | 0.545±0.03 |
| | 100 | 233.65±3.13** | 2.43±0.04** | 0.546±0.01 |
| **Ih** | 10 | 285.60±3.35*** | 2.44±0.02*** | 0.481±0.01*** |
| | 100 | 321.42±2.74*** | 2.23±0.01*** | 0.505±0.03** |
| **Ii** | 10 | 234.23±2.79** | 2.44±0.02*** | 0.547±0.04 |
| | 100 | 244.46±3.43*** | 2.35±0.02*** | 0.557±0.03 |
| **Ij** | 10 | 317.90±3.67*** | 2.51±0.03*** | 0.484±0.01*** |
| | 100 | 345.19±2.68*** | 2.26±0.02*** | 0.471±0.02*** |

Values are expressed as mean ± SD (Number of animals, n=6), *, **and *** indicates not significance, (*) p<0.05, (**)p<0.01 and (***) p<0.001vs.control (Newman-Keuls multiple comparison test).

hypercholerimic and hyperkalemic diuretics which supports the claim about the isatin derivatives being used as a potent diuretics.

### 3.5. Evaluation of Diuretic Index and Electrolytic Excretion Index

Table **4** shows the diuretic and electrolyte index ($Na^+$, $K^+$, ${}^-Cl$) of all the groups. All the treated groups show the higher diuretic and electrolyte index compared with control group.

## 4. DISCUSSION

Diuretics relieve pulmonary congestion and peripheral edema. These agents are useful in reducing the syndrome of volume overload, including orthopnea and paroxysmal nocturnal dyspnoea. They increase plasma volume and subsequently venous return to the heart. This decreases cardiac work load, oxygen demand and plasma volume, thus decreasing blood pressure. Thus diuretics play an important role in hypertensive patients. Diuretics are modulating the volume and composition of body fluids in variety of clinical conditions like hypertension, heart failure and cirrhosis. Diuretics alone or in combination with other antihypertensive drugs are considered to be more effective than the calcium channel blockers and angiotensin converting enzymes inhibitors as the first line treatment of hypertension. It also helps in the prevention of one or more forms of cardiovascular diseases in high risk patients with hypertension[34]. The seventh report guidelines issued in the United States by the Joint National Committee on prevention, evaluation, and treatment of high blood pressure, and England and Wales, the National Institute for Health and Clinical Excellence guidelines recommend the use of low dose diuretics as first line pharmacological treatment for high blood pressure [34]. The diuretic

**Table 4: Effect of Isatin Derivatives on Urine Output Index and Electrolytic Excretion Index in 5th hour of Urine Collection**

| Treatment | Dose(mg/kg) | Diuretic index | $Na^+$ | $K^+$ | $Cl^-$ |
|---|---|---|---|---|---|
| **Control** | 25 ml of 0.9% NaCl | 1.00 | 1.00 | 1.00 | 1.00 |
| **Urea** | 1 g/kg | 1.94 | 1.06 | 1.12 | 1.06 |
| **Furosemide** | 5 | 3.50 | 1.64 | 1.88 | 1.48 |
| **Ia** | 10 | 1.31 | 1.02 | 1.06 | 1.02 |
| | 100 | 1.38 | 1.02 | 1.08 | 1.02 |
| **Ib** | 10 | 2.58 | 1.38 | 1.47 | 1.20 |
| | 100 | 2.94 | 1.54 | 1.76 | 1.37 |
| **Ic** | 10 | 1.36 | 1.02 | 1.07 | 1.02 |
| | 100 | 1.41 | 1.03 | 1.08 | 1.02 |
| **Id** | 10 | 2.50 | 1.04 | 1.20 | 1.06 |
| | 100 | 2.77 | 1.13 | 1.20 | 1.31 |
| **Ie** | 10 | 1.50 | 1.03 | 1.08 | 1.02 |
| | 100 | 2.33 | 1.11 | 1.16 | 1.12 |
| **If** | 10 | 2.50 | 1.29 | 1.16 | 1.12 |
| | 100 | 2.83 | 1.44 | 1.57 | 1.32 |
| **Ig** | 10 | 1.31 | 1.02 | 1.06 | 1.02 |
| | 100 | 1.37 | 1.02 | 1.07 | 1.03 |
| **Ih** | 10 | 2.52 | 1.32 | 1.38 | 1.17 |
| | 100 | 2.86 | 1.44 | 1.65 | 1.37 |
| **Ii** | 10 | 1.41 | 1.03 | 1.08 | 1.03 |
| | 100 | 2.20 | 1.06 | 1.15 | 1.09 |
| **Ij** | 10 | 2.63 | 1.47 | 1.50 | 1.30 |
| | 100 | 3.02 | 1.60 | 1.81 | 1.41 |

Diuretic index, urine volume of test group/urine volume of control group; Na+ index, sodium excretion in test group/sodium excretion in control group; K+ index, potassium excretion in test group/potassium excretion in control group; Cl− index, chloride excretion in test group/chloride excretion incontrol group.

therapy is also useful in the treatment of edema, hypocalcaemia, hepercalceuria, diabetes insipidus andacute renal failure [35]. In the present study all the isatin derivatives (**Ia-Ij**) show increase the excretion of urine output over a period of 5 hr. From the above result significant difference in urinary excretion followed by diuretic action and diuretic activity was observed. All isatin derivatives shows good to little diuretic activity and only these three derivatives (**Ia, Ic** and **Ig**) show nil diuretic activity but they also increased the excretion of $Na^+$, $^-Cl$ accompanied with $K^+$ significantly as compared to control. Furosemide acts by inhibiting electrolyte reabsorption in the thick ascending limb of loop of henle [36].The increased excretion of urine output indicated by isatin derivatives and furosemide were statistically significant compared with control. After the administration of different doses of test compounds the high dose produced highest urine volume ,electrolyte output and the low dose produce significant urine output, electrolyte excretion but was less when compared with furosemide and more or less similar to that of urea. The isatin derivatives contain different substitutions at 5th position ($NO_2$, Cl, $CH_3$ and H) because of these substitutions of bromo and nitro group in the 5th position of isatin increased the activity due to increased lipophilicity [37]. From this observation our isatin derivatives also possess good activity. From the reported data in the present work indicates that isatin derivatives showed good to nil diuretic activity in comparison with control and we observed that our derivative is an effective hypernatremic, hypercholermic and hyperkalemic diuretics which are similar to conventional diuretics. From the observations isatin derivatives had similar diuretic spectrum to the furosemide.

## 5. CONCLUSION

The present work indicated that isatin derivatives showed good diuretic activity. From the above result we observed that the isatin derivatives showed hypernatremic, hypercholerimic and hyperkalemic,

natriuretic, saluretic and CAI activity which is similar to the diuretic spectrum of furosemide. However, a detailed study is required to know the mechanism of action of the potent molecules.

## ACKNOWLEDGEMENT

The authors are grateful to Secretary, Viswambhara Educational Society, Warangal for providing the necessary facilities to carry out the study.

## REFERENCES

[1] Agunu A, Abdurahman EM, Andrew GO, Muhammed Z. Diuretic activity of the stem-bark extracts of *Steganotaenia araliacea* hochst [Apiaceae]. J Ethanopharmacol 2005; 96: 471-75. http://dx.doi.org/10.1016/j.jep.2004.09.045

[2] Schappert SM. National Ambulatory Medical Care Survey: Summary. Vital Health Stat 1994; 13: 78-80.

[3] Ulmann A, Sayegh F, Clavel J, Lacour B. Incidence of lithiasic recurrence after a diuretic therapy, alone or combined with treatment by a thiazide diuretic or phosphorus. Press Med 1984; 13: 1257-60.

[4] Gupta S, Neyses L. Diuretic usage in heart failure: a continuing conundrum in 2005. Eur Heart J 2005; 26: 644-49. http://dx.doi.org/10.1093/eurheartj/ehi784

[5] Morganti A. Should a diuretic always be the first choice in patients with essential hypertension? The case for no. J Am Soc Nephrol 2005; 16: S70-S73. http://dx.doi.org /10.1681/ASN.200411096

[6] Soleimani M, Barone S, Xu J, Shull GE, Siddiqui F, Zahedi K, Amlal H. Double knockout of pendrin and Na-Clco-transporter (NCC) causes severe salt wasting, volume depletion, and renal failure. Proc Natl Acad Sci USA 2012; 109: 13368-73. http://dx.doi.org/10.1073/pnas.1202671109

[7] Yasar S, Lin FM, Fried LP, Kawas CH, Sink KM, DeKosky ST, Carlson MC. Diuretic use is associated with better learning and memory in older adults in the Ginkgo Evaluation of Memory Study. Alzheimers Dement 2012; 8: 188-95. http://dx.doi.org/10.1016/j.jalz.2011.03.010

[8] Glover V,Reveley MA, Sandler MA. Monoamine oxidase inhibitor in Human urine. Biochem Pharmacol 1980; 29: 467-70. http://dx.doi.org/10.1016/0006-2952(80)90534-1

[9] Glover V, Halket JM,Watkins PJ,Clow A,Goodwin BL, Sandler M. Isatin: Identity with the purified endogenous monoamine oxidase inhibitor tribulin. J Neurochem 1988; 51: 656-59. http://dx.doi.org/10.1111/j.1471-4159.1988.tb01089.x

[10] Kumar NS, Pradeep T, Jani G, Silpa D, Kumar BV. Design, synthesis, and antimicrobial screening of novel pyridyl-2-amidrazoneincorporated isatin mannichbases. J Adv Pharm Technol Res 2012; 3: 57-61. http://dx.doi.org/10.4103/2231-4040.93559

[11] Aboul-Fadl T, Abdel-Aziz HA, Abdel-Hamid MK, Elsaman T, Thanassi J, Pucci MJ. Schiff bases of indoline-2, 3-dione: potential novel inhibitors of Mycobacterium. Molecules 2011; 16: 7864-79. http://dx.doi.org/10.3390/molecules16097864

[12] Mondal P, Jana S, Balaji A, Ramakrishna R, Kanthal L. Synthesis of Some New Isoxazoline Derivatives of Chalconised Indoline 2-one as a Potential Analgesic, Antibacterial and Anthelmimtic Agents. J Young Pharm 2012; 4: 38-41. http://dx.doi.org/10.4103/0975-1483.93574

[13] Barcelos RP, de Lima Portella R, Lugokenski TH, da Rosa EJ, Amaral GP, Garcia LF, *et al*. Isatin-3-$N^4$- benzyl thiosemicarbazone, a non-toxic thiosemicarbazone derivative protects and reactivates rat and human cholinesterases inhibited by methamidophos *in vitro* and *in silico*. Toxicol In Vitro 2012; 26: 1030-39. http://dx.doi.org/10.1016/j.tiv.2012.04.008

[14] Aboul-Fadl T, Radwan AA, Attia MI, Al-Dhfyan A, Abdel-Aziz HA. Schiff bases of indoline-2, 3-dione (isatin) with potential antiproliferative activity. Chem Cent J 2012; 6: 49. http://dx.doi.org/10.1186/1752-153X-6-49

[15] Havrylyuk D, Zimenkovsky B, Vasylenko O, Gzella A, Lesyk R. Synthesis of new 4-thiazolidinone- pyrazoline- and isatin-based conjugates with promising antitumor activity. J Med Chem 2012; 55: 8630-41. http://dx.doi.org/10.1021/jm300789g

[16] Praveen C, Ayyanar A, Perumal PT. Practical synthesis, anticonvulsant, and antimicrobial activity of N-allyl and N-propargyldi(indolyl)indolin-2-ones. Bioorg Med Chem Lett 2011; 21: 4072-77. http://dx.doi.org/10.1016/j.bmcl.2011.04.117

[17] Venkateshwarlu E, Venkateshwar Rao J, Umasankar K, Dheeraj G. Study of anti-inflammatory, analgesic and antipyretic activity of novel isatin derivatives. Asian J Pharm Clin Res 2012; 5: 187-90.

[18] Nataraj KS, Venkateshwara Rao J, Jayaveera KN. Diuretic activity of some novel Isatin Derivatives. J Pharm Res 2010; 3: 863-65.

[19] Raghunandan N, Devi MA, Sriram D, Sarangapani M. Synthesis and pharmacological screening of some new derivatives of (Z)-[2-(2-substituted benzalamino-4-yl) hydrazono]-indolin-2-ones. Der Pharma Chemic 2010; 2: 96-106.

[20] Sarangapani M, Reddy VM. Synthesis and screening of isatin-3-$N^2$-(alkoxyethyloxy) acetylhydrazones. Indian drugs 1999; 36: 357-62.

[21] John J, Bobade AS, Khadse BG. Synthesis of some new triazole ring systems from 2, 4–Disubstitued thiazole. Indian J Hetero Chem 2001; 10: 295-98.

[22] Twaij HAA, Elisha EE, Al-Jeboory AA. Screening of Iraqi Medicine Plants for Diuretic Activity. Indian J Pharmac 1985; 73-76.

[23] Stanic G, Samarzija I, Blazevic N. Time dependent diuretic response in rats treated with Juniper berry preparations. Phytotherap Res 1998; 12: 494-97. http://dx.doi.org/10.1002/(SICI)1099-1573(199811)12:7<494::AID-PTR340>3.0.CO;2-N

[24] Lipschitz WL, Hadidian Z, Kerpesar A. Bioassay of diuretics. Pharmacol Ex Therap 1943; 79: 97-110.

[25] Mukherjee PK, Pal M, Saha K, Saha BP. Diuretic activity of extract of the rhizomes of *Nelumbonucifera Gaertn*. Phytotherapy Res 1996; 10: 424-25. http://dx.doi.org/10.1002/(SICI)1099-1573(199608)10:5<424::AID-PTR857>3.0.CO;2-3

[26] Murugesan T, Manikandan L, Suresh KB, Pal M, Saha BP. Evaluation of diuretic potentials of *Jussiaea Suffruticosa* Linn extracts in rats. Indian J Pharma Sci 2000; 62: 150-51.

[27] Indian Pharmacopoeia. Publications and Information Directorate (CSIR), New Delhi, India 1996; 2: 689.

[28] Al-Ali M, Wahbi S, Twaij H, Al-Badr A. Tribulusterrestris: Preliminary study of its diuretic and contractile effects and comparision with *Zeamays*. J Ethanopharmacol 2003; 85: 257-260. http://dx.doi.org/10.1016/S0378-8741(03)00014-X

[29] Alvarez ME, Maria AOM, Villegas O, Saad JR. Evaluation of diuretic activity of the constituents of *Clematis monteridensis* Spreng in Rats. Phytotheraphy Res 2003; 17: 958-60. http://dx.doi.org/10.1002/ptr.1268

[30] Mamun MM, Billah MM, Ashek MA, Ahasan MM, Hossai MJ, Sultana T. Evaluation of diuretic activity of *Ipomoea aquatica* in mice model study. J Med Sci 20039; 3: 395-400.

[31] Somova LI, Shode FO, Ramanan P, Nadar A. Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from *Oleaeuropaea,* subspecies Africana leaves. J Ethanopharmacolo 2003; 84: 299-305. http://dx.doi.org/10.1016/S0378-8741(02)00332-X

[32] Amuthan A, Bharathi C, Bairy KL, Sudhakar, Prakash M. Evaluation of diuretic activity of *Amaranthus spinoosus* Linn. aqueous extract in Wistar rats. J Ethanopharmacolo 2012; 140: 424-27. http://dx.doi.org/10.1016/j.jep.2012.01.049

[33] Vogel GH, Eds. Drug Discovery and Evaluation: Pharmacological Assays. Germany: Springer Verlag 2002; pp. 324-25.

[34] Boger-Megiddo I, Heckbert SR, Weiss NS, McKnight B, Furberg CD, Wiggins KL, *et al*. Myocardial infarction and stroke associated with diuretic based two drug antihypertensive regimens: population based case-control study. BMJ 2010; 340: c103. http://dx.doi.org/10.1136/bmj.c103

[35] Krumlovsky FA, del Greco F. Diuretic agents. Mechanisms of action and clinical uses. Post grad Med 1976; 59: 105-10.

[36] Shinkawa T, Yamaski F, Notsu T, Nakakuki M, Nishijima K, Yoshitomi K, Imai M. Loop and distal action of novel diuretics. Euro J Pharmacol 1993; 238: 317-25. http://dx.doi.org/10.1016/0014-2999(93)90863-D

[37] Smitha S, Pandeya SN, Stables JP, Ganapathy S. Anticonvulsant and sedative-hypnotic activities of N-Acetyl/Methyl isatin derivatives. Sci Pharm 2008; 76: 621-36. http://dx.doi.org/10.3797/scipharm.0806-14

Received on 01-01-2013 Accepted on 18-03-2013 Published on 30-04-2013

DOI: http://dx.doi.org/10.6000/1927-5951.2013.03.02.5