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EVALUATION OF DIURETIC AND ANTIDIABETIC ACTIVITIES OF ESCULIN IN WISTAR RATS

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ABSTRACT

Diabetes mellitus is a common endocrine disorder, characterized by hyperglycaemia resulting from defects in insulin secretion action or both. 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. Ayurvedic herbs are relatively low cost, more suitable and have negligible side-effects than synthetic oral anti-hyperglycaemic agents and diuretics. The objective of the present study was to evaluate the diuretic and anti-diabetic activity of Esculin on experimental animals. The selected male Wistar albino rats were divided into different groups and treated with Esculin at 100 and 200 mg/kg for evaluation of diuretic activity with normal saline (25 ml/kg) loaded animals and for evaluation of antidiabetic activity, Streptozotocin-Nicotinamide-induced diabetic rats were selected. The urine volume (in ml) and content of Na^+, K^+ & Cl^- were measured in the urine of rats at 5th hour and estimated blood glucose levels in rats. All data were expressed as means ± SD. Dunnett's test and one-way ANOVA test was used to compare the mean values of test groups and control. The Esculin at 200 mg/kg shows significant diuretic ($P<0.001$), significantly the urinary excretion of Na^+ ($P<0.001$), K^+ ($P<0.01$), Cl^- ($P<0.001$) when compared with control group and significant ($P<0.01$) reduction in blood glucose level at 200 mg/kg on 7th day. The data suggests that the Esculin may have produced its diuretic and antidiabetic activity via multiple mechanisms.

KEY WORDS

Anti-diabetic activity, Diuretic activity, Esculin, Flame photometry.

INTRODUCTION

Diuretics are the agents which causes increase in excretion of urine. These drugs generally used in the treatment of oedema, hypertension, and congestive heart failure (CHF), nephritis, toxaemia and other UTI disorders. Diuretics are also used in the treatment of pulmonary congestion and play vital role in pregnancy and premenstrual tension [1]. Schappert reported more than 45 million peoples treated by diuretic alone in cardiac patients [2]. Presently, in market, synthetic diuretics are available which are having significant side effects. These synthetic diuretics significantly inhibit K^+ secretion and leads to K^+ retention. A natural source serves as

an additional source for the development of new diuretic agents because of their biological activity [3].

Diabetes mellitus is a multi-factorial disorder characterized by hyperglycaemia resulting from an increased hepatic glucose production, diminished insulin secretion and impaired insulin action. It is a disease of worldwide significance and increasing its prevalence without any plateau [4]. In addition to adverse effects, drug treatments are not always satisfactory in maintaining normal levels of blood glucose and avoiding late stage diabetic consequences [5]. However, many medicinal plants have been provided a potential source of Antidiabetic and diuretic principles and were widely used. A large number of clinical trials were carried out to test the hypoglycaemic activity of plants and pure chemical compounds were isolated from the crude extract of plants [6]. One such compound is Esculin and the present study is to evaluate the diuretic and anti-diabetic activity of Esculin.

Esculin (6, 7-dihydroxycoumarin-6-o-glucoside) is a coumarin derivative found in *Aesculus hippocastanum* L. (Horse-chestnut). Their seeds have long been used to treat inflammatory and vascular problems and also against kidney stones and stomach pain. Esculin is known to be a 5- and 12-lipoxygenase inhibitor and to inhibit the production of leukotrienes and 5-hydroxyeicosatetraenoic acid through the lipoxygenase pathway [7]. In 2007, Zhao used the dopamine-induced cytotoxicity model in human euroblastoma SH-SY5Y cells to demonstrate that Esculin inhibited dopamine-induced caspase-3 cleavage and decreased cell death, over production of ROS, morphological changes of nuclei and damage to antioxidant enzymes [7]. Esculin scavenges hydroxyl radicals and inhibits lipid peroxidation in the rat liver [8]. Esculin also was found to have gastro protective effect. It is also used in the manufacturing of pharmaceuticals with venotonic. Other actions include capillary protection and the inhibition of enzymes like hyaluronidase and collagenase. It improved skin vasculature and is effective in the management of cellulitis [9].

Materials and Methods

Animals

Male Wistar albino rats of 150-200 gm were selected for this study, procured from Mahaveer enterprises, Hyderabad, India and was acclimatized for one week under standard laboratory conditions (25±2 °C, relative humidity of 45 to 55% and 12:12 hr light and dark cycle), fed with standard rodent pellet diet and water. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC NO: 1047/Ac/07/CPCSEA).

Drugs / Dose / Route of administration

Esculin was purchased from Yucca enterprises (Mumbai, India), Streptozotocin and Nicotinamide were procured from SISCO laboratories (Mumbai, India), glibenclimide and furosemide were obtained as a gift sample from Natcopharma (Hyderabad, India). Glucose kits were purchased from Coral laboratories. It was administered at doses of 100 and 200 mg/kg, *p.o.*

DIURETIC ACTIVITY

Experimental design

Thirty animals were used in this study. The animals were divided into 7 groups. Each group consisting of 6 animals (n = 6).

Group I: Received vehicle (0.1% sodium CMC) orally and served as control

Group II: Received Urea (1gm/kg)

Group III: Treated with standard drug (Furosemide 10 mg/kg)

Group IV: Treated with 100 mg/kg of ESCULIN

Group V: Treated with 200 mg/kg of ESCULIN

Group VI: Treated with Furosemide+ Test-I 20+100

Group VI: Furosemide+ Test-II 20+200

Immediately after administration of the drugs, rats were individually placed in metabolic cages with total withdrawal of food and water *ad libitum.* The urine was collected from individual animal at 5thhr [10, 11]. The urine volume (ml) was measured and assayed for Na^+, K^+ and Cl^- concentrations [12-14]. 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 [15]. The instrument was calibrated with standard solutions containing different concentrations of sodium and potassium [16].

Measurement of Urinary Excretion, Diuretic Activity and Diuretic Action [17, 18]

The urinary excretion, diuretic activity and diuretic action of the control, urea, furosemide and Esculin treated groups were calculated from the following equations:

$$\text{Urinary Excretion} = \frac{\text{Total Urinary Output } (V_0)}{\text{Total Liquid Administered } (V_1)} \times 100$$

$$\text{Diuretic Action} = \frac{\text{Urinary Excretion in Test Group (UEt)}}{\text{Urinary Excretion in Control Group (UEc)}}$$

$$\text{Diuretic Activity} = \frac{\text{Diuretic Action of Urea (DAu)}}{\text{Diuretic Action of Drug (DAt)}}$$

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 [19].

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 [20].

DIABETIC ACTIVITY

Hypoglycaemic activity

To determine the hypoglycaemic activity of the drug, normoglycaemic rats were fasted for 18 hours. They were divided into three groups of six rats each.

Group I served as normal control and received orally 0.1% sodium CMC (vehicle).

Group II animals were fed with test drug of Esculin at oral dose of 100 mg/kg body weight in vehicle.

Group III animals were fed with test drug of Esculin at oral dose of 200 mg/kg body weight in vehicle.

The samples of blood were obtained zero, second, third, and fourth hour of the treatment. The blood glucose levels were determined using a glucometer. [21, 22]

Streptozotocin– Nicotinamide induced diabetes (STZ-NIC)

The animal model of type-2 diabetes mellitus (NIDDM) was induced in overnight fasted rats by administering a single dose of freshly prepared solution of Streptozotocin (60mg/kg b.wt *i.p* in 0.1mol / L) cold citrate buffer (pH 4.5), 15 min after the administration of Nicotinamide (120 mg / kg b. wt, *i.p*) Streptozotocin treated animals were allowed to drink 5% glucose solution overnight to

overcome drug induced hypoglycaemia. After 7 days of development of diabetes, rats with fasting blood glucose of more than 200 mg/dl were considered as diabetic and were used for further experimentation [23].

Group I: Received vehicle (0.1% sodium CMC) orally and served as control

Group II: Served as Diabetic control (STZ-NIC)

Group III: Treated with standard drug (Glibenclimide 10 mg/kg)

Group IV: Treated with 100 mg/kg of Esculin

Group V: Treated with 200 mg/kg of Esculin

After the administration of drugs blood samples were collected from the retro-orbital plexus of rats, by inserting a fine capillary gently. Plasma samples were analysed for glucose by GOD/POD method [24].

Statistical analysis

Results were expressed as mean ± SD. One-way analysis of variance (ANOVA) was carried out, and the statistical comparisons among the groups were performed with Dunnett's test and *P* value <0.05 were considered as statistically significant.

RESULTS

Effects on Urine Output and Diuretic Activity

Urea, furosemide, and Esculin with both doses increases the urine output significantly (*P*<0.001) when compared with control group. Urine volume was increased significantly with increased Dose with 100 to 200mg/kg of Esculin at 5th hr [Table 1]. Esculin 200 mg/kg dose were showed greater diuretic activity than 100 mg/kg dose. 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.

Effects on Electrolyte Excretion

The diuretic responses with its electrolyte excretion potency of the Esculin were highly moderate in comparison with control animals. The Esculin at doses of 100 and 200 mg/kg showed increase in Na^+, Cl^- excretion, accompanied by the excretion of K^+.

The 200 mg/kg doses of Esculin enhance significantly the urinary excretion of Na^+ (*P*<0.001), K^+ (*P*<0.01), Cl^- (*P*<0.001) when compared with control group [Table 2].

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

Table 2 shows the natriuretic, saluretic and CAI activity after oral administration of Esculin, urea, furosemide and control groups. Esculin at both doses (100 and 200 mg/kg) showed marked saluretic, natriuretic and CAI activity comparable to control group. The natriuretic ratio values>2 indicate favourable natriuretic activity. With decreasing the CAI ratio values <0.8 slight to strong CAI activity could assumed. From the above results it can be suggested that the Esculin 200 mg/kg are an effective hypernatremic, hypercholerimic and hyperkalemic diuretics which supports the claim about the isatin derivatives being used as a potent diuretics.

Evaluation of Diuretic Index and Electrolytic Excretion Index

Table 3 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.

DIABETIC ACTIVITY

Hypoglycaemic activity

The oral administration of Esculin at 100 and 200 mg/kg to normal rats shows significant (*P*<0.01) reduction in blood glucose was observed with only 200 mg/kg at 4th hr when compared with control. Oral administration of vehicle did not change significant level of basal blood glucose. The standard glibenclimide were significantly (*P*<0.001) decreased the blood glucose and the data was represented in Table 4.

Effect on blood glucose levels in streptozotocin-nicotinamide induced diabetic rats

The antidiabetic activity of Esculin showed significant (P <0.01) reduction in blood glucose level at 200 mg/kg on 7th day and 100 mg/kg shows significant (P <0.05) fall in blood glucose compared with diabetic control. Glibenclimide shows significant (P<0.001) antidiabetic activity. There was a significant antidiabetic activity at the 4th h for both 100 and 200 mg/kg doses [Table 5].

DISCUSSION

This study was undertaken to evaluate the diuretic and anti-diabetic activity of Esculin in rats. The currently available drug regimens for management of diabetes mellitus and diuretics have certain drawbacks and therefore there is a need to find safer and more effective diuretic and anti-diabetic drugs.

Diuretics relieve pulmonary congestion and peripheral oedema. These agents are useful in reducing the syndrome of volume over load, including orthopnoea 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 [25]. 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 [26]. 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 [27]. The diuretic therapy is also useful in the treatment of oedema, hypocalcaemia, hepercalceuria, diabetes insipidus and acute renal failure [28].

Diuretic activity of Esculin in normal rats possesses a potent diuretic activity. The diuretic potency was comparable to that of standard drug furosemide. Here, the drug increases the total volume of urine and excretion of Na^+ and K^+. The diuretic effects of both concentrations of the drug are indicated by increase in both water excretion and excretion of Na^+ and K^+. The active principles responsible for the diuretic effect of the drug have not yet been elucidated but as the drug is a flavonoid, the effect may be produced by stimulation of regional blood flow or initial vasodilatation or by producing inhibition of tubular reabsorption of water and anions, the result in both cases being diuresis.

Anti-hyperglycaemic activity of plant derived products needs extensive research as the number of diabetic patients is continuously on the rise and according to WHO projections; it will be the single largest non-communicable disease worldwide by the year 2025 with the largest diabetic population in India. Management of diabetes with the agents devoid of any side effects is still a challenge to the medical system. This concern has led to an increase and demand for natural products with anti-hyperglycaemic activity having fewer side effects.

The present study discussed about the anti-diabetogenic effect of Esculin. Streptozotocin-Nicotinamide induced diabetes in a dose

dependent fashion. Streptozotocin injection resulted diabetes mellitus, which may be due to destruction of β cells of Islets of Langerhans. Fasting blood glucose levels of untreated diabetic rats were significantly higher than those in normal rats. Over production of glucose by means of excessive hepatic glycogenolysis and gluconeogenesis is one of the fundamental bases of hyperglycaemia in diabetes mellitus.

Diabetes induction caused significant (P<0.01) hyperglycaemia. In this study, the test drug Esculin at different doses produce a significant fall in the blood glucose level in both normal and diabetic rats in a dose dependent manner and this was evident 4 hr after the administration of the drug. On the other hand Glibenclimide caused significantly more hypoglycaemia in comparison with the test drug (200 mg/kg). The mechanism of this hypoglycaemic effect of the drug is not elucidated in this study. Further studies will be focused on the determination of the mechanism(s) of action.

Among the two doses of test drug, 200 mg/kg dose showed significant anti-hyperglycaemic effect. The proposed mechanism of action may be by promoting regeneration of β-cells or by protecting the cells in pancreas from destruction, by restricting glucose load as well as by promoting unrestricted endogenous insulin action and further effect β-cells to release insulin and activate the insulin receptors to absorb the blood sugar. Regeneration of islet β-cells following destruction by Streptozotocin may be the primary cause of the recovery.

Table 1: Dose Response Diuretic Activity of Esculin in Normal Rats at 5th hour by Oral administration

Groups	Dose (mg/kg)	Volume of urine (ml)	Urinary excretion (Vo/Vt) X 100	Diuretic action UEt/UEc	Diuretic activity DAt/DAu
Control	25ml of 0.9% NaCl	0.62±0.11	13.32	-	-
Urea	1g/kg	1.32±0.13***	28.81	2.16	-
Furosemide	20	2.33±0.15***	51.12	3.84	1.77
Test-I	100	1.71±0.17***	38.00	2.85	1.31
Test-II	200	1.96±0.19***	43.50	3.27	1.51
Furosemide+ Test-I	20+100	2.36±0.14***	59.00	4.42	2.04
Furosemide+ Test-II	20+200	2.90±0.18***	70.73	5.31	2.45

Values are expressed as mean ± SD of six rats in each group in comparison with the control group. Vo = Total urinary output; Vt = Total fluid input; UEt = Urinary excretion in test group; UEc = Urinary excretion in control group; DAt = Diuretic action test group; DAu= Diuretic action of the urea . *** P <0.001, ** P <0.01, * P < 0.05, Significant compared to control analysed by one-way ANOVA followed by Dunnett's test.

Table 2: Electrolytes Excretion (mMol/L), Saluretic, Natriuretic and CAI Activity of Esculin in Normal Rats at 5th hour by Oral Administration

Groups	Dose (mg/kg)	Na^+	Cl^-	K^+	$Na^+ + Cl^-$	Na^+/K^+	$Cl^-/Na^+ + K^+$
Control	25 ml of 0.9% NaCl	98.58±1.12	121.92±1.13	53.58±1.12	220.50±2.22	1.83±0.05	0.80±0.01
Urea	1g/kg	102.72±1.13***	126.72±1.14**	59.69±1.14**	229.44±2.10[1]**	1.72±0.06	0.78±0.01
Furosemide	20	138.06±1.14***	165.72±1.15***	97.38±1.15***	303.78±2.59***	1.41±0.05	0.70±0.02
Furosemide+ Test-I	---	142.24±1.21***	155.42±1.12***	50.24±1.82	297.66±2.33***	2.83±0.04***	0.80±0.01
Furosemide+ Test-II	---	150.14±1.81***	160.42±1.22***	53.34±1.32	310.56±3.03***	2.81±0.05***	0.78±0.02
Test-I	100	105.20±1.15***	134.58±1.18**	57.62±1.17**	239.78±2.09**	1.82±0.05	0.78±0.02
Test-II	200	128.46±1.16***	150.84±1.19***	63.63±1.19**	279.32±2.77***	2.01±0.06***	0.82±0.02***

Values are expressed as mean ± SD (Number of animals, n=6), * *P* <0.001, ** *P* <0.01, * *P* < 0.05, Significant compared to control analysed by one-way ANOVA followed by Dunnett's test.**

Table 3: Effect of Esculin on Urine Output Index and Electrolytic Excretion Index in 5th hour of Urine Collection

Group	Dose(mg/kg)	Diuretic index	Na^+	K^+	Cl^-
Control	25ml of 0.9% NaCl	1.00	1.00	1.00	1.00
Urea	1g/kg	2.16	1.04	1.11	1.03
Furosemide	20	3.83	1.40	1.81	1.35
Test-I	100	2.85	1.06	1.07	1.10
Test-II	200	3.26	1.30	1.18	1.23

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 in control group.

Table 4: Hypoglycaemic effect of Esculin in normal rats

Group	Dose (mg/kg)	Blood Glucose Levels (mg/dl)					
		Pre-treatment (hours)	Post treatment (hours)				
		0	1	2	4	6	8
Control		118.33±4.50	120.00±2.62	118.61±7.80	115.35±2.62	110.10±1.76	115.52±2.53
Glibenclimide	10	118.32±3.12	111.85±5.26 (5.4±3.4)	81.16±4.72** (31.3±4)	67.16±3.10*** (43.2±3.1)	77.16±2.63** (34.7±0.6)	96.81±11.30 (18.2±8.9)
Test-I	100	123.81±2.32	116.62±2.50 (5.7±2.5)	112.62±2.75 (9.0±2.4)	110.50±2.13 (10.7±1.9)	112.00±1.42 (9.5±1.1)	116.34±5.84 (6.0±3.3)
Test-II	200	120.34±1.80	113.65±3.52 (5.5±1.8)	102.30±7.62* (14.9±6.8)	83.82±1.90** (30.3±1.5)	108.12±8.60 (10±7.7)	111.80±3.32 (7±2.4)

Values are expressed as mean ± SD (Number of animals, n=6), * *P* <0.001, ** *P* <0.01, * *P* < 0.05, Significant compared to control analysed by one-way ANOVA followed by Dunnett's test.**

Table 5: Effect of Esculin on fasting blood glucose levels in diabetic rats

Group	Dose(mg/kg)	Blood Glucose Levels (mg/dl)					
		Pre-treatment (hours)	Post treatment (hours)				
		0	1	2	4	6	8
Diabetic Control		237.50±13.30	231.62±16.00	233.00±11.14	226.16±8.22	223.51±7.94	212.00±11.21
Glibenclimide	10	245.33±20.62	223.11±10.11 (9.61±7.11)	195.16±7.70** (20.29±3.91)	157.10±13.80*** (36.51±5.32)	197.80±19.70* (19.82±9.93)	226.12±7.11 (8.51±5.62)
Test-I	100	233.32±20.62	220.00±19.93 (5.27±2.12)	208.51±16.82 (10.51±3.22)	196.11±17.00* (15.81±2.82)	210.52±15.14 (9.62±2.71)	222.10±14.80 (4.62±2.91)
Test-II	2000	248.63±12.92	232.12±8.13 (6.51±3.12)	213.00±7.20* (14.10±4.30)	177.00±11.23** (28.81±1.40)	202.50±7.50 (18.40±2.32)	217.00±5.71 (12.63±2.52)

Values are expressed as mean ± SD (Number of animals, n=6), * *P* <0.001, ** *P* <0.01, * *P* < 0.05, Significant compared to control analysed by one-way ANOVA followed by Dunnett's test.**

CONCLUSION

In conclusion administration of Esculin produced study provide a quantitative basis to explain the potent diuretic activity warrants future detailed investigation as a promising diuretic agent.

Administration of Esculin produced a significant reduction of glucose levels in STZ-induced diabetic rats. However, comprehensive chemical and pharmacological researches are required to find out the exact mechanism of this drug for its anti-diabetogenic effect. However, it seems promising that if these data will be validated in the future clinical trials, Esculin may offer an alternative treatment for type II Diabetes.

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Conflicts of interest

There are no conflicts of interest.

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