

ORIGINAL ARTICLE

Evaluation of Diuretic and Antidiabetic Activity of Esculin

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ABSTRACT

Diuretics are the agents which cause increase in excretion of urine. Diabetes mellitus (DM) is a multi-factorial disorder characterized by hyperglycemia resulting from an increased hepatic glucose production and diminished insulin secretion. The present study was undertaken to study the diuretic and anti-diabetic activity of esculin. Esculin was found to have potent diuretic activity in normal rats and the potency was comparable to that of standard drug furosemide. The diuretic effects of both concentrations of the drug were indicated by increase in water, sodium and potassium excretion. Esculin also at different doses produced a significant fall in the blood glucose level in both normal and diabetic rats in a dose-dependent manner which was evident 4 h after the administration of the drug. Among the two doses of test drug (100 and 200 mg/kg), the later dose showed significant anti-hyperglycemic activity.

Keywords: Anti-diabetic activity, Diuretic activity, Esculin, Flame photometry

Diuretics are the agents which cause increase in excretion of urine. These drugs are generally used in the treatment of edema, hypertension, and congestive heart failure (CHF), nephritis and toxemia. 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 [2]. Presently, most synthetic diuretics are available in market have significant side effects. These synthetic diuretics significantly inhibit K^+ secretion which 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 hyperglycemia resulting from an increased hepatic glucose production, diminished insulin secretion and impaired insulin action. It is a disease of worldwide significance and its prevalence is increasing 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].

Esculin (6,7-dihydroxycoumarin-6-o-glucoside) is a coumarin derivative found in *Aesculus hippocastanum* L. (Horse-chestnut). Chemical structure of esculin has been shown in Fig 1. The plant 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 [6]. In 2007, Zhao used the dopamine-induced cytotoxicity model in human neuroblastoma 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 properties. 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].

Many medicinal plants have been provided a potential source of antidiabetic and diuretic principles. A large number of clinical trials were carried out to test the hypoglycemic activity of plants and pure chemical compounds were isolated from the crude extract of plants [8]. One such compound is esculin and the present study is to evaluate its diuretic and anti-diabetic activity.

MATERIALS AND METHODS

Chemicals

Esculin was purchased from Yucca enterprises (Mumbai, India). Streptozotocin and nicotinamide were procured from SISCO laboratories (Mumbai, India). Glibenclamide and furosemide were obtained as a gift sample from Natcopharma (Hyderabad, India). Glucose kits were purchased from Coral laboratories. All the chemicals were obtained from SS Pharma, Hanamkonda and they were of analytical grade quality.

Animals

Male Wister rats aged four months (180 ± 10 g) used for the present study were procured from Mahaveer enterprises, Hyderabad, India. The animals were housed in polyacrylic cages ($38 \text{ cm} \times 23 \text{ cm} \times 10 \text{ cm}$) with no more than six animals per cage, at an ambient temperature of $18 \pm 2^\circ\text{C}$ with 12-h light and dark cycle. Rats have free access to standard chow diet and water *ad libitum*. The maintenance and the handling of animals were performed according to the rules and regulations of Institutional Animal Ethical Committee (IAEC).

Diuretic activity

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 two groups were treated with esculin (100 and 200 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 5th hour [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 ($\text{N}/50$) 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:

$$\text{Urinary excretion} = \frac{\text{Total urinary output (Vo)}}{\text{Total liquid administered (V1)}} \times 100$$

$$\text{Diuretic action} = \frac{\text{U.E. in test group (UEt)}}{\text{U.E. in control group (UEc)}}$$

$$\text{Diuretic activity} = \frac{\text{Diuretic action of drug (DAT)}}{\text{Diuretic action of urea (DAu)}}$$

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 $\text{Cl}^-/(\text{Na}^+ + \text{K}^+)$ 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].

Anti-diabetic activity

Streptozotocin-nicotinamide-induced diabetes

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 60 mg/kg b.w.i.p in 0.1 mol/L cold citrate buffer ($\text{pH } 4.5$), 15 min after the intraperitoneal administration of 120 mg/kg Nicotinamide. Streptozotocin-treated animals were allowed to drink 5% glucose solution overnight to overcome drug-induced hypoglycemia. After 7 days of development of

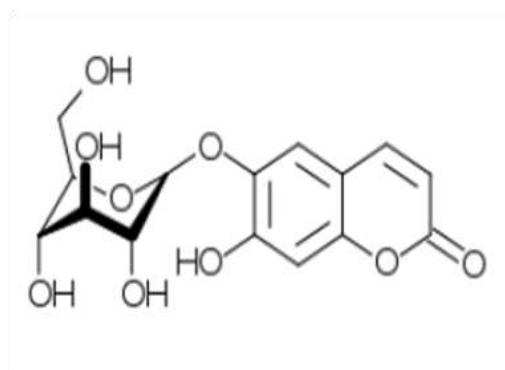


Fig 1. Chemical structure of esculin

Table 1. Diuretic activity of esculin

Treatment groups	Dose (mg/kg)	Volume of urine (ml)	Urinary excretion (v ₀ /v ₁)*100	Diuretic action UE _t /UE ₀	Diuretic activity Da _t /DA ₀
Control	25 ml of 0.9% NaCl	0.62 ± 0.11	13.32	-----	-----
Urea	1 g/kg	1.32 ± 0.13***	28.81	2.16	-----
Furosemide	20 mg/kg	2.33 ± 0.15***	51.12	3.84	1.77
Test-1	100 mg/kg	1.71 ± 0.17***	38.00	2.85	1.31
Test-2	300 mg/kg	1.96 ± 0.19***	43.50	3.27	1.51

Data represents mean ± S.D. (n = 5). ****p* < 0.001, ***p* < 0.05, Significant compared to diabetic control analyzed by one-way ANOVA followed by Dunnett's test.

Table 2. Electrolytic parameters of urine after esculin treatment

Treatment	Na ⁺ (mMol/L)	Cl ⁻ (mMol/L)	K ⁺ (mMol/L)	Na ⁺ + Cl ⁻	Na ⁺ /K ⁺	Cl ⁻ /Na ⁺ + K ⁺
Control	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	102.72 ± 1.13***	126.72 ± 1.14**	59.69 ± 1.14**	229.44 ± 2.11**	1.72 ± 0.06	0.78 ± 0.01
Furosemide	138.06 ± 1.14***	165.72 ± 1.15***	97.38 ± 1.15***	303.78 ± 2.59***	1.41 ± 0.05	0.70 ± 0.02
Test-1	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-2	128.46 ± 1.16***	150.84 ± 1.19***	63.63 ± 1.19**	279.32 ± 2.77***	2.01 ± 0.06***	0.82 ± 0.02***

Data represents mean ± S.D. (n = 5). ****p* < 0.001, ***p* < 0.01, **p* < 0.05, Significant compared to diabetic control analyzed by one-way ANOVA followed by Dunnett's test.

Table 3. Effect of esculin on urine output index and electrolytic excretion index in 5th h of urine collection

Treatment group	Diuretic index	Na ⁺	K ⁺	Cl ⁻
Control	1.00	1.00	1.00	1.00
Urea	2.16	1.04	1.11	1.03
Furosemide	3.83	1.40	1.81	1.35
Test-1	2.85	1.06	1.07	1.10
Test-2	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.

diabetes, rats with fasting blood glucose of more than 200 mg/dL were considered as diabetic and were used for further experimentation [21].

Six rats were formed into one group; five such different groups of rats were formed and used for studying the effects of the selected drug. The first group was normal untreated group and received 0.1 % sodium CMC orally. The second group was the diabetic untreated group which received streptozotocin and nicotinamide as per their respective doses and also received 0.1 % sodium CMC. The third group was standard-treated group which received streptozotocin and nicotinamide along with 5 mg/kg b.w of glibenclamide orally. The fourth and fifth groups were test groups which received 100 and 200 mg/kg b.w of esculin respectively orally. 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 analyzed for glucose by GOD/POD method [22].

Statistical analysis

All the data were expressed as mean ± SD (n = 6) and evaluated by one way analysis of variance (ANOVA), employing Dunnett's test and values of *p* < 0.05 were considered as statistically significant.

RESULTS

One way ANOVA followed by Dunnett's test showed a significant diuretic activity for 100 and 300 mg/kg doses of esculin when compared to that of control. Various parameters estimated were urine volume, urinary excretion rate and diuretic activity. Table 1 shows these parameters and their comparison with that of Urea and the Furosemide treated groups (Table1).

Effect of esculin on the urinary excreted ions was estimated. Urine collection was done after the 5th h of

Table 4. Hypoglycemic activity 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 ± 4.5	120 ± 2.6	118.6 ± 7.8	115.3 ± 2.6	110.1 ± 1.7	115.5 ± 2.5
Std	10	118.3 ± 3.1	111.8 ± 5.2 (5.4 ± 3.4)	81.16 ± 4.7** (31.3 ± 4)	67.16 ± 3.1*** (43.2 ± 3.1)	77.16 ± 2.6** (34.7 ± 0.6)	96.8 ± 11.3 (18.2 ± 8.9)
Test1	100	123.8 ± 2.3	116.6 ± 2.5 (5.7 ± 2.5)	112.6 ± 2.7 (9.0 ± 2.4)	110.5 ± 2.1 (10.7 ± 1.9)	112 ± 1.4 (9.5 ± 1.1)	116.3 ± 5.8 (6.0 ± 3.3)
Test2	150	120.3 ± 1.8	113.6 ± 3.5 (5.5 ± 1.8)	102.3 ± 7.6* (14.9 ± 6.8)	83.8 ± 1.9** (30.3 ± 1.5)	108.1 ± 8.6 (10 ± 7.7)	111.8 ± 3.3 (7 ± 2.4)

Values are Mean ± SD, n = 6 in each group. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ when compared with vehicle-treated group (Dunnett's test). Parenthesis indicates the % reduction of glucose values.

Table 5. Anti-diabetic activity of esculin

Group	Dose (mg/kg)	Blood Glucose Levels (mg/dL)					
		Pre-treatment (h)	Post-treatment (h)				
		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
Glibenclamide	10	245.33 ± 20.62	223.11 ± 10.11 (9.61 ± 7.11)	195.1 ± 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)
Esculin	100	233.3 ± 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)
Esculin	150	248.63 ± 12.92	232.1 ± 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 Mean ± SD, n = 6 in each group. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ when compared with diabetic control group (Dunnett's test). Parenthesis indicates the % reduction of glucose values.

drug administration. The results showed that the excretory rates of Cl^- , Na^+ and K^+ was increased significantly in both test groups when compared with that of the control group. Similarly, the saluretic, natriuretic and diuretic activities were also increased (Table 2).

Effect of esculin on the diuretic and electrolytic indices was calculated with respect to the control group. The test- 2 group showed almost similar activity as the standard group (Table 3).

Hypoglycemic activity was observed in the normal rats and the blood glucose levels were recorded. In the post treatment evaluation, the blood glucose levels were reduced significantly at the 4th h in the test group 2 when compared with that of the control (Table 4). Then, there was a gradual increase in the blood glucose levels. Antidiabetic activity of esculin was done in diabetic rats using glibenclamide as standard drug. There was a

significant antidiabetic activity at the 4th h for both 100 and 150 mg/kg doses of the test drug (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 edema. These agents are useful in reducing the syndrome of volume over load, 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 [23]. 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 [24]. The seventh report guidelines issued in the United States of America by the Joint National Committee on Prevention, Evaluation, and Treatment of High Blood Pressure, and in England and Wales by the National Institute for Health and Clinical Excellence recommend the use of low dose diuretics as first line pharmacological treatment for high blood pressure [25]. The diuretic therapy is also useful in the treatment of edema, hypocalcaemia, heparcalcuria, diabetes insipidus and acute renal failure [26].

Esculin posses a potent diuretic activity in normal rats. 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 sodium and potassium. 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, initial vasodilatation or by producing inhibition of tubular reabsorption of water and anions.

Anti-hyperglycemic activity of plant derived products needs extensive research as the number of diabetic patients is continuously on the rise. 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-hyperglycemic 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 hyperglycemia in diabetes mellitus. Diabetes induction caused significant hyperglycemia ($p < 0.001$). 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 h after the administration of the drug. On the other hand, glibenclamide caused significantly more hypoglycemia in comparison with the test drug (150 mg/kg). The mechanism of this hypoglycemic effect of the drug is not elucidated in this study. Further studies will be focused on the determination of the mechanism(s) of action.

The present study was undertaken to evaluate the anti-diabetic activity of esculin in streptozotocin-induced diabetic rats. Among the two doses of test drug, 150 mg/kg dose showed significant anti-hyperglycemic effect. The proposed mechanism of action may be by promoting regeneration of β -cells or by protecting the cells in the pancreas from destruction, by restricting glucose load as well as by promoting unrestricted endogenous insulin action and further effective β -cells to release insulin and activating 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.

In conclusion, 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 antidiabetogenic effect. 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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