

Research Article

Evaluation of Hypoglycemic Activity of Ethanolic Extract of *Murraya koenigii* Stem in Alloxan Induced Albino Rats Involving Possible Antioxidant Mechanism

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INTRODUCTION

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, keto-acidosis or a non-ketotichyper osmolar state may develop and lead to stupor, coma, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made. The long-term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease. Several path genetic processes are involved in the development of diabetes. These include processes which destroy the beta cells of the pancreas with consequent

insulin deficiency, and others that result in resistance to insulin action. The abnormalities of carbohydrate, fat and protein metabolism are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin.

TYPES OF DIABETES MELLITUS

Diabetes mellitus commonly known as sugar diabetes or disease can be divided into 3 parts

1. Type 1 Diabetes Mellitus
2. Type 2 Diabetes Mellitus
3. Gestational Diabetes Mellitus

MATERIALS AND METHODS

PLANT

The fresh stem of *Murrayakoenigii* was harvested and in the month of July 2011 from the garden of Padmavathi college of pharmacy, periyanaahalli, Tamilnadu India. The plant was authenticated by Prof.P.Jayaraman.Ph.D. Director, National Institute of Herbal Sciences Chennai. The plant material was carefully washed with tap water and left to dry at room temperature.

ANIMALS

Adult male and female rats of wisteralbino strain weighing between 200-300gm, were obtained from the Laboratory Animal House, Sasthra University Thanjavur. They were kept in polypropylene cages and allowed to get acclimatized to a standard laboratory diet. The animals were adapted to laboratory condition prior to the

experiments and constant room temperature at 22°C–24°C with 12 hour day and night cycle. Feed and drinking water were provided ad libitum. The studies were performed with the approval of Institutional Animal ethics committee (IAEC) following the guide lines of CPCSEA.

CHEMICALS

Stem of *Murraya kornigii* Padmavathi college of pharmacy garden, Alloxan Scientific Laboratories Dharmapuri, Glibenclimide scientific laboratories dharmapuri, Estimation kits Mercury Scientific, Salem. All the reagents and chemicals used in the present study were of analytical grade.

EXTRACTION OF CRUDE DRUGS

The successive extraction of powdered Stem was done by using Soxhlet apparatus, For extraction, 250gm of powdered stem was packed in thimble containing watmann filter paper and extracted with ethanol 90% (60°C-70°C) in soxhlet apparatus for the period till all the crude substances were extracted. The extract thus obtained was concentrated with the help of rotatory vacuum evaporator.

PREPARATION OF EXTRACT

Suspension of ethanolic extract was prepared in 0.5% carboxymethyl cellulose using Tween 20 (0.2% v/v) as suspending agent. The extract was administered in a dose of 300 mg/kg respectively to alloxan induced diabetic Wister rats.

EXPERIMENTAL DESIGN

Experimental animals are divided in five groups each groups having six animals (albino rat-Wister strain)

Group I. Normal group Received 1 ml of saline and served as control. Once a day orally for 15 days by using an intra gastric tube.

Group II. Positive control group received alloxan 120mg/kg body weight Administered through intra-peritonially

Group II1. Standard group received glibeclamide 10mg /kg body weight once a

day orally for 15 days by using an intragastric tube.

Group IV. Received Ethanol extraction of *Murrayakoenigii* stem 300 mg/kg body weight once a day orally for 15 days by using an intra gastric tube

After inducing alloxan rats were fasted overnight and blood was withdrawn from the retero orbital puncture 1st on the 15th day of induction of diabetes. Blood glucose level, total cholesterol, triglycerides, Body weight were determined for all the four groups

HYPOGLYCEMIC STUDY IN (WISTER STRAIN) ALBINO RAT

Diabetes was induced by intraperitoneal injection of alloxan monohydrate (5% w/v) in physiological saline at a dose of 120 mg/ kg body weight The rats were fasted for 48 hours before inducing diabetes with alloxan animals were injected by intraperetonal route with alloxan of 120mg/kg per body weight in 0.9% freshly prepared saline solution which was sterilised before use in autoclave. control animals received saline solution alone. Alloxan induced animals were allowed to drink 5%glucose solution to overcome drug induced hypoglycaemia for 2 days. Alloxan treated rats were divided into a group .the treatment was started on the 4th day after induced diabetes and this is considered as the first day of the treatment.

STATISTICAL ANALYSIS

The quantitative measurements were made on six animals in each group and the values were expressed as Mean \pm SEM data obtained was subjected to one way (ANOVA). Followed by Turkey Multiple comparisons test and $p < 0.01^*$, 0.001^{**} were consistent

RESULTS AND DISCUSSION

THE PERCENTAGE YIELD=10.5%w/w

The percentage yield of ethanolic extract of *Murrayakoenigii* stem was found to be:

Total amount of crud drug used=200gm.

Amount obtained as ethanolic extract= 10.5%w/w.

Table 1

PLANT NAME	PART USED	METHOD OF EXTRACTION	PERCENTAGE YIELD
<i>Murrayakoenigii</i>	Stem	Soxhlet apparatus	10.5%w/w

PHYTOCHEMICAL TESTS FOR ETHANOLIC EXTRACT OF *Murrayakoenigi* stem
The Ethanolic extract of *Murrayakoenigii* stem subjected to phyto chemical screening

shows the presence of alkaloids, tannins and phenolic compounds
The results are as follows:

Table 2: Preliminary phytochemical results

Phytochemical constituents	Ethonal extract
Alkaloids	+
Glycoloids	-
Tannins and phenolic compounds	+
Steroids	-
Saponins	-
Flavonoids	-
Proteins, amino acids	-
Carbohydrats	-

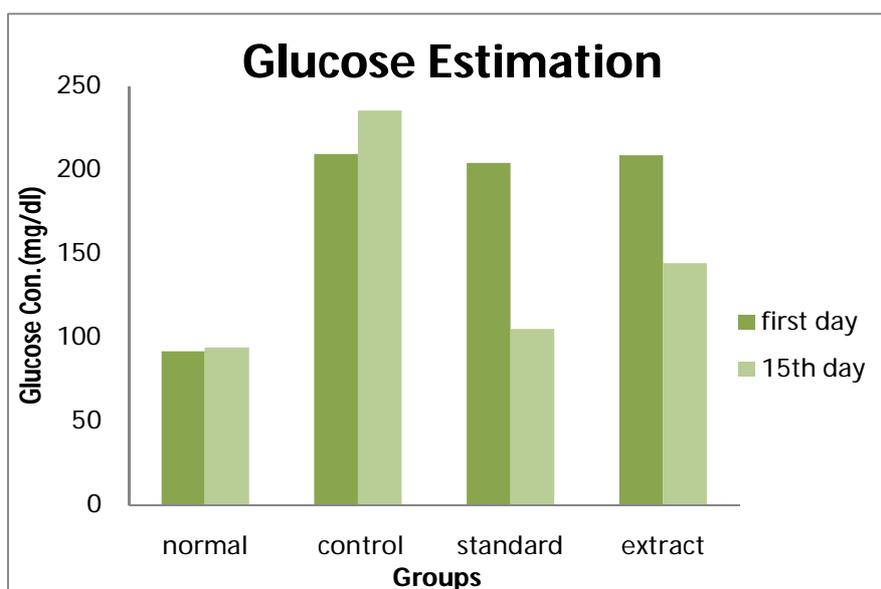
(+) indicates presence (-) indicates absence

ESTIMATION OF GLUCOSE

Table 3

Number of days	Normal	Positive control	standard	Extract
1 st day	91.56±4.93	209.46±8.16	204.05±10.10*	208.67±10.01
15 th day	94.14±4.80	235.39±11.45	104.86±11.45**	144.41±6.79**

Values are expressed as mean ± SEM., (n=6), *P < 0.01 significant, **p < 0.001 Highly Significant



Graph 1: Glucose concentration Vs treated groups

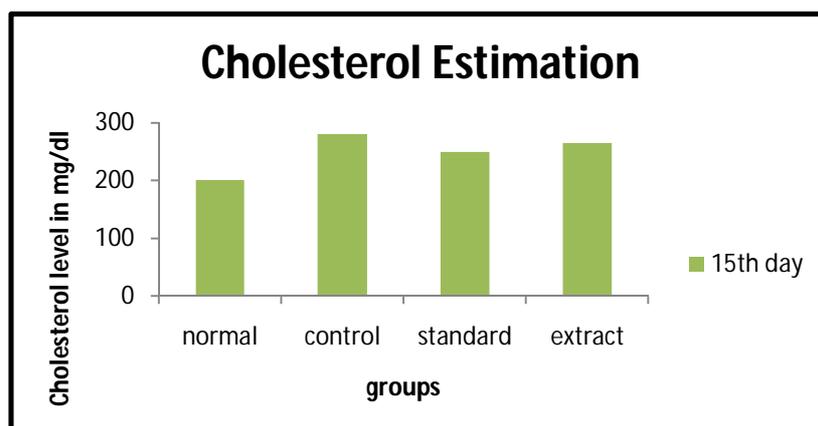
Table 3 shows, alloxan induced Diabetes, by damaging the insulin secreting cells of the pancreas leading to hyperglycemia (Szeuldelski: 2001). Administration of alloxan 120mg/kg (i.p) lead to elevation of fasting blood glucose levels, along with significant decrease in body weight over a period of 15 days and it was partially restored or improved upon administration of *Murrayakoenigis* stemethanolic extract significantly decreased the elevated blood glucose level comparison to untreated diabetic. Wister strain albino rats after 15 days of daily treatment with extract led to fall in blood glucose level by 40-50% respectively.

ESTIMATION OF TOTAL CHOLESTEROL

Table 4

Normal	199.1±2.5
Diabetic	270.3±3.1
Glebenclamide	255.33±1.4**
Extract	265.5±0.8**

Values are expressed as mean ± SEM., (n=6),
*P < 0.01 significant, **p < 0.001 Highly Significant



Graph 2: Cholesterol Vs Treated groups

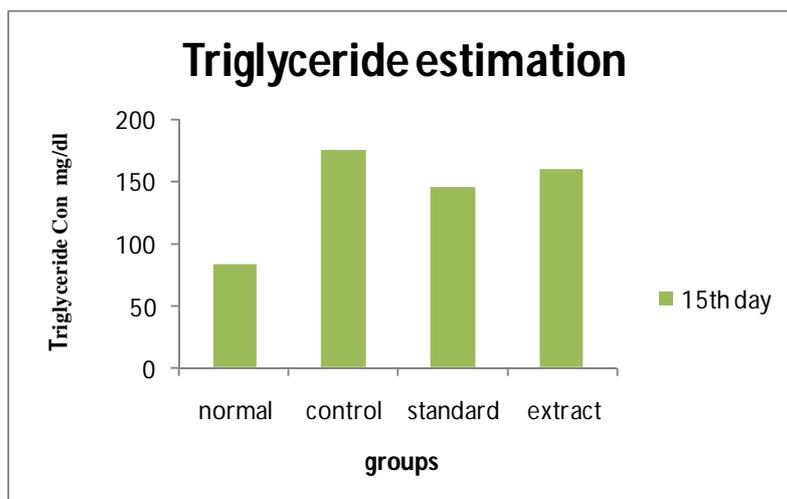
The Cholesterol, significantly reduces in Ethanol extract, treated diabetic rats respectively, ethanolic extract shows significance.

ESTIMATION OF TRIGLYCERIDES

Table 5

Normal	96.6±2.6
Diabetic	183.8±4.4
Gibanclimedel	122.6±5.4**
Extract	106±5.1**

Values are expressed as mean ± SEM., (n=6),
*P < 0.01 significant, **p < 0.001 Highly Significant



Graph 3: Triglycerides Concentration Vs Treated Groups

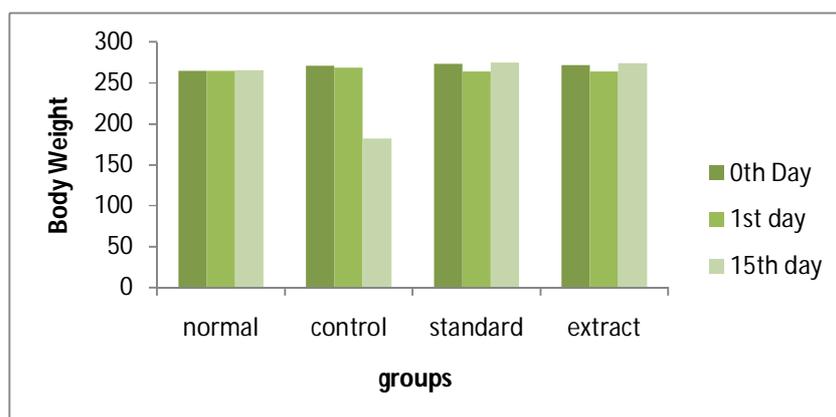
The triglycerides, also significantly reduces in Ethanol extract, treated diabetic rats, respectively, ethanolic extract shows significance.

Body weights of alloxan induced diabetic rats after treatment with *Murrayakoenigii* stem extract

Table 6

Groups	Before diabetes induction	After inducing diabetes 1 st Day	15 th Day
Normal	265.30±18.50	265.40±17.60	266.80±13.55
Control	271.60±12.30	269.50±13.90	182.00±13.55
Glibenclamide	273.30±18.40	264.60±23.30	275.0±15.90**
Extract	272.10±14.80	264.50±15.50	274.90±5.10**

Values are expressed as mean ± SEM., (n=6), *P < 0.01 significant, **p < 0.001 Highly Significant



Graph 4: Body Weight Vs Treated Groups

DISCUSSION

Murrayakoenigii. belongs to Rutaceae family. There are approximately fourteen

global species available in India it is more popular due to its large spectrum of medicinal properties and also because of the use of its leaves for centuries as a

natural flavouring agent in various curries and food items. It is more or less deciduous shrub or a small tree up to 6m in height.

However no scientific investigation has so far been conducted on the stem for its anti-diabetic activity. In view of alleged antihypoglycemic potential of *Murrayakoenigii*, I have investigated effect of ethanolic extract on fasting blood sugar level serum bio-chemical analysis in alloxan induced diabetic rats. Diabetes mellitus a common heterogenous metabolic syndrome, is prevalent all over the world and has been projected to become one of the world's main disablers and killers within next 25 years (King, et al, 1998). The results of present study indicates that *Murrayakoenigii* stem Ethanol extract shows significant to reduce the Blood glucose level, Total Cholesterol, Triglycerides, Body weight.

The Percentage yield of the stem extract using Soxhlet apparatus was found to be 10.5%. Phytochemical studies for the ethanolic extract reveals the presence of alkaloids, tannins and phenolic compounds. In Table 3 the blood glucose levels were compared to the standard and control, the extract shows hypoglycemic activity which is less significant than the standard and positive control.

In Table 4 the cholesterol levels were compared to the standard and the positive control, the extract reduces the cholesterol level which is less significant compared to the standard and more significant than the positive control.

In table 5 ethanolic extract significantly reduces the triglyceride levels compared to the positive control.

From Table 6 the body weights seemed to be increased slightly compared to the control and less significant than the standard.

CONCLUSION

In the present study dried powdered stem of *Murrayakoenigii* was subjected to

extraction with Ethanol (90%). Results of preliminary photochemical screening lead to the conclusion that Tannins, phenolic & alkaloids are present as phytochemical constituents of stem extract. Results of pharmacological screening have led to the conclusion that alkaloids fraction exhibited prominent anti-hypoglycaemic activity in Wistar strain albino rats similarly and insignificant inhibitory action against diabetes. Extract of Ethanol was evaluated for its possible anti-diabetic properties by standard *in vitro* models. Ethanol extract showed significant anti-hypoglycaemic activity when compared with standard drug. The Ethanol extract reduced the Serum, total Cholesterol, Triglycerides, increased Body weight level in Diabetic induced rats.

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