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Research Article

Report on Antidiabetic, Diuretic and Analgesic Activities of Methanolic Extract of Leaves of *Strychnos colubrina L.*an Endangered Medicinal Plant

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ABSTRACT

Strychnos colubrina L. belongs to family Loganiaceae was known as snake wood tree.The methanolic extracts from Strychnos colubrina L. leave collected from different provinces in Penchalakona of Nellore district were prepared by decoction and maceration with methanol and evaluated for their antidiabetic, diuretic and analgesic activities. Methanolic extracts from *Strychnos colubrina* L. leaves (SCM) were evaluated for anti diabetic effect in Streptozocin (STZ) induced diabetes in rats. The blood sugar levels were analysed as indices of diabetes. 200 mg/kg b.w. of the extract showed a greater reduction in blood glucose level which was comparable to glibenclamide. To find out diuretic efficacy, SCM of leaves were administered to experimental rats orally at doses of 100 and 200 mg/kg and compared with Furosemide (20 mg/kg,o.p) as the standard. The rats treated with SCM of leaves in a dose of 200 mg/kg shown near similar urine output and electrolytes excretion when compared to the respective control. The analgesic activity of SCM of leaves is estimated using tail flick in mice. Results demonstrated that SCM of leaves exhibited a potent dose-dependent analgesic activity in all tested models for analgesia. This report could be used for medicinal and pharmaceutical exploration in the future.

Keywords: Methanolic extract, snake wood tree, Streptozocin, glibenclamide, Furosemide, Lipschitz test, tail flick method.

INTRODUCTION

Plants form the basis of various traditional medicines throughout the world. Phyto medicines derived from green plants have been used by about 65% of the world's population. All though there are many ways to control diseases because of their secondary complications, phytomedicines are preferred due to minimum side effects and easy usage and preparation. The use of medicines and food supplements derived from plant has been increased in recent years. Botanists, microbiologists, Ethno pharmacologists, and chemists are searching the earth for phytochemicals and drugs which could be used for the treatment of many stubborn infectious diseases in a green and natural way. Today we have more concern with lifestyle disease like depression, cancer and heart troubles caused by stress and faulty nutrition. Because these diseases have emotional components, there is a growing concern that English medicine is largely unable to cure them all but it offers a temporary relief from pain and disease symptoms. So there is an urgent need for alternative therapy, to cover a good health for all. Herbal therapy will be one of the practices to overcome the illness (Gupta,and Chime, 2000).Many traditional and local plants are used for the treatment of diabetes mellitus throughout the world (Marles and Fransworth, 1995). Ayurvedic medicines are called the elixirs of life and they have an important role in improving health and disease treatment from ancient days to this modern time. Plant medicines provide good means for the treatment of many diseases that are either absent or incurable in other systems of medicine. Herbal plants, which form the backbone of alternative medicine, have in the last few decades been the subject for very intense pharmacological studies; this has been brought by the agreement of the value of medicinal plants as an important source of new compounds of therapeutic values in the drug development. Due to these facts over the past twenty years, a considerable revival of interest in the use of herbal medicine in the world has come up.

Diabetes mellitus is a metabolic disorder initially characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin action, insulin secretion, or both (Barcelo and Rajpathak, 2001). If the glucose level in the blood remains high over a long period of time, this can result in long-term damage to organs, such as the kidneys, nerves, heart, liver, eyes and blood vessels. Complications in some of these organs can lead

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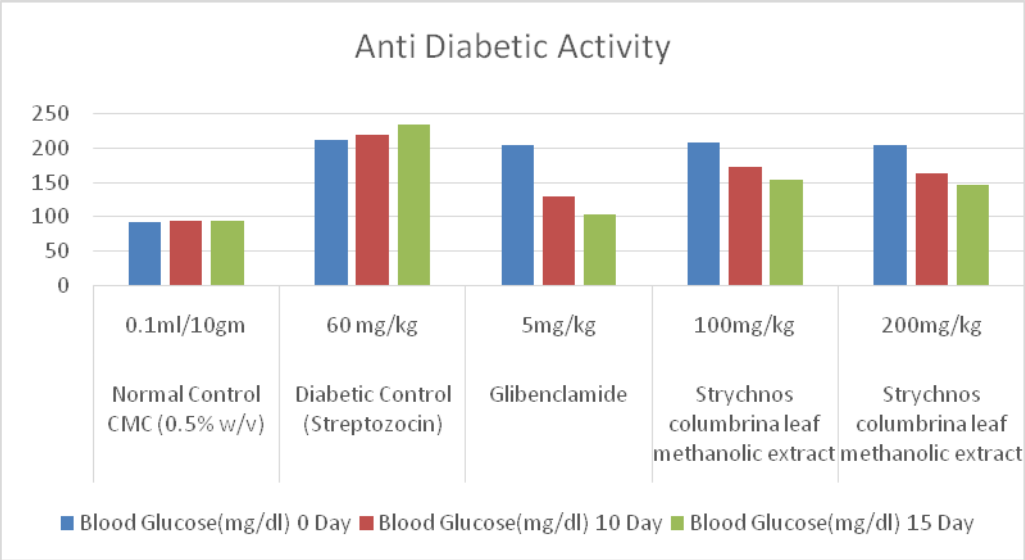
Table 1: Results of Antidiabetic activity.

S.No	Treatment	Dose	Blood Glucose(mg/dl)		
			0 day	10 day	15 day
1	Normal Control CMC (0.5% w/v)	0.1ml/10gm	92.34	93.12	93.58
2	Diabetic Control (Streptozocin)	60 mg/kg	213.04	220.72	235.34
3	Streptozocin (60 mg/kg) + Standard drug-Glibenclamide (5mg/kg, p.o).	5mg/kg	205.78	130.3	104.24
4	*Strychnos colubrina*leaf methanolic extract	Streptozocin(60 mg/kg) + Test 1 (100 mg/kg)	208.14	173.8	153.94
		Streptozocin(60 mg/kg)+ Test 2 (200mg/kg)	204.14	163.36	145.92



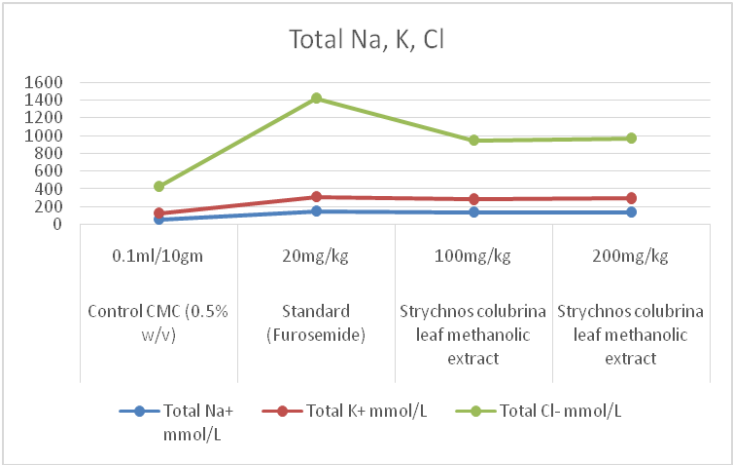
Graph: 1 showing antidiabetic activity.

Table 2: Results of Diuretic activity.

S.No	Treatment groups	Dose (mg/kg)	Urine volume (ml/24hrs)	Total Na^+ mmol/L	Total K^+ mmol/L	Total Cl^- mmol/L	Na^+/ K^+ Ratio
1	Control CMC (0.5%w/v)	0.1ml/10gm	9.78	61.3	69.2	299.6	0.89
2	Standard (Furosemide)	20mg/kg	34.5	150.4	158.2	1113.66	0.946
3	*Strychnos colubrina* leaf methanolic extract	100mg/kg 200mg/kg	28.06 30.76	139 142.06	145.2 150.84	661.6 681.96	0.952 0.936

to death (Pari and Saravanan, 2004). Diuretics, also called water pills, are drugs used to increase the quantity of water and salt excreted from the body as urine. There are three types of prescription diuretics. They are often prescribed to help treat high blood pressure, but they are used for other conditions as well. These medicines, when used for longer periods, may show side effects. An analgesic is a medicine that takes away the physical pain but these medicines, when used on a long-term basis, will give bad impact on vital organs like kidneys etc.

In this research work, I have examined the unexplored properties of medicinal plant *Strychnos colubrina* scientifically for its antidiabetic, diuretic and analgesic activities. In spite of its extensive use in folk, ayurvedic, herbal and tribal medicine the above properties of leaves of *Strychnos colubrina L.* were not properly reported. Hence the present study has been taken for this analysis.Various plant parts of *Strychnos colubrina L.*are being used in the Indian Ayurvedic system for the cure of various diseases.In Ayurveda, it is called Kupilu-lataa, Kuchilaalataa. Whereas in Maharashtra the folklore name



Graph: 2 showing amounts of Na, K, Cl, ions excreted for diuretic activity.

Table 3: Results of Analgesic activity.

S.No	Treatment	Dose	Reaction time in seconds			
			Basal	30	60	90
1	Vehicle CMC (0.5%w/v)	0.1ml/10gm	4.14	4.16	4.17	4.15
2	Standard (Pentazocin)	30mg/kg	4.21	9.05	11.05	12.42
3	*Strychnos colubrina* leaf methanolic extract	100mg/kg	4.2	5.2	5.67	6.35
		200mg/kg	4.25	6.31	7.13	8.96

is Kaajar-vel. In Malayalam, its common name is Vallikanjiram and Modirakanjiram.

This taxon is found in Western Peninsular India covering parts of Karnataka and Kerala; it is so farrecorded at penchalakona of Nellore district in Eastern PeninsularIndia. Theexistence of the plant also recorded in Chittoor District particularly at Brahmadevudigundam (Mamandur), Kambakkam hills, Ambakkam, Sadhumalammakona, (Madhava Chetty, *et al.*, 2011). First Red list of Medicinal plants of Andhra Pradesh, India – Conservation Assessment and Management planning is reported it as an Endangered Species in Andhra Pradesh, India[1]. Woody climbers; very bitter, with tendrils[2]. *Strychnos colubrina L.*Wood is also used for malarial fever and cutaneous eruptions and Rheumatism. The tribal people used the root of this plant for the snake bite. Fruit is used to prevent Mania. Leaves and roots are boiled in oil and applied to rheumatic swellings. Root is purgative, febrifugal, anthelmintic. The roots, wood, bark, and seeds contain alkaloids (bark wood), consisting of brucine and strychnine. Beta-sitosterol has been reported in the plant[3]. The Order exhibits febrifuge and tetanic properties. Itis very rich in alkaloids- brucine, bakankosine, curine, curarine, gelsemidine, gelsemine, gelseminine. gelsemoidine, koumine, kouminicint, kouminidine, kouminine, protucurarine, protocuridine, rrotocurine, sempervine, sempervirine, spigeline, struxine, strychnicine. Strychnine, tubocurarine. The presence of a glucoside, aesculin, has been recorded[3].

MATERIALS AND METHODS

Plant collection and identification

The plant material (leaves) of *Strychnos colubrina L.* of family Loganiaceae, were collected from Penchalakona forest of the Eastern Ghats, Nellore district, Andhra Pradesh in July 2017. The plant was identified with the help of' The flora of Nellore district' and 'Flora of Madras'[4,5]. The leaves of *Strychnos colubrina L.* plant material were shade dried, powdered and stored in desiccators until further use. All the specimens at different leaf stages were documented in the form of herbarium sheets which have been deposited in the Herbarium, Department of Botany, Vikramasimhapuri University, P.G Centre, and Kavali, Andhra Pradesh, India.

The powdered material was then extracted using solvents methanol in the ratio 1:10 using Soxhlet apparatus. (Khan *et al.*, 1988). The solvent was evaporated under reduced pressure in the rotatory evaporator to get methanol extract. The components were separated to the solvents based on their polarity. The extract was subjected to the rotary evaporator at 40^{0C} to remove the excess solvent from the extract. After extracting all colouring material, the solvent was removed by evaporating in a water bath, which gave rise to a solid isolate of extract used for determining antidiabetic, diuretic and analgesic activities.

Determination of the Ant diabetic activity of Strychnos colubrina methanolic(SCM) leaf extracts

Healthy Wistar rats (150 to 200 gm) of either sex were selected. Before and during the experiment, rats were fed with the standard diet. After randomization into various groups and before initiation of the experiment; the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Blood samples were collected through tail vein puncture method at weekly intervals over a period of 28 days. Animals described as fasting, which were deprived of food and water for 18 hours.

Table 4: Maximum possible effects of drugs in tail flick model of analgesia in rats.

S.No	Drugs	Dose	% Maximum possible effect in seconds		
			30	60	90
1	Vehicle CMC (0.5%w/v)	0.1ml/10gm	-----	----	---
2	Standard (Pentazocin)	30mg/kg	44.85	63.39	76
3	*Strychnos colubrina* leaf methanolic extract	100mg/kg	9.25	15.87	23.22
		200mg/kg	19.16	26.79	50.63

After fasting, DM was induced by IP injection of Streptozocin (STZ) at a dose of 60 mg/Kg. The animals were given to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. After 72 hrs, STZ-treated animals were treated as diabetic when the fasting blood glucose levels observed above 200 mg/dL with glucosuria[6]. Fasting blood glucose was measured by glucose oxidase-peroxidase (GOD-POD) method (Trinder, 1969) in mg/dl using a digital glucometer (Braun Omnitest REZ, Germany).

5 rats in each group which were as follows:

Group I: Normal control (saline).

Group II: Streptozocin treated control (60 mg/kg.ip).

Group III: Streptozocin (60 mg/kg) + Standard drug-Glibenclamide (5mg/kg, p.o).

Group IV: Streptozocin (60 mg/kg) + Test 1(100 mg/kg)

Group V: Streptozocin (60 mg/kg) + Test 1(200 mg/kg)

The vehicle, Test samples, and Glibenclamide were administered once daily for 15 days from the day of induction. Blood was drawn from the tail tip of rat and the blood glucose level was estimated on 0, 10^{th} and 15^{th} day of experiment with the help of glucometer using strip method.

Determination of the Diuretic activity of Strychnos colubrina(SCM) leaf extracts

The diuretic activity in Wistar rats was studied by the Lipschitz Test[7] (1943). The test is based on water and sodium excretion in test animals and compared to rats treated with a high dose of Furosemide.

Four groups of Wistar rats were used to evaluate the diuretic activity of methanolic extract of leaves of *Strychnos colubrina*(SCM)by using metabolic cages. The group I served as normal control given vehicle (CMC 0.5% w/v in normal saline), group II with Furosemide (20 mg/Kg, p.o), Groups III and IV with 100 mg/kg, 200 mg/ doses ofSCM respectively.Immediately after the treatment with the standard and test all the rats were hydrated with saline (15 ml/kg) and placed in the metabolic cage, specially designed to separate urine and feces and kept at 21°C±0.5°C.

Estimation of Urinary Electrolytes

Urine electrolytes (sodium, potassium, and chloride) were determined by Ion Selective Electrode method as described by the user manual of the biochemical kits (NRI Technologies, Malleswaram, Bengaluru)

5 rats in each group which were as follows:

Group I: Normal control (CMC 0.5% w/v in normal saline).

Group II: Furosemide (20 mg/kg, p.o).

Group III: Test 1(100 mg/kg)

Group IV: Test 1(200 mg/kg)

The total volume of urine collected after 24 hrs was measured at the end. During this time no water and food were given to rats. Different parameters like total urine volume and amount of sodium, potassium, and chloride in the urine were measured and calculated respectively. The ratio of Sodium to Potassium ions is also estimated.

Determination of the Analgesic activity of Strychnoscolubrinamethanolic (SCM)leaf extracts:

Analgesia was measured using a modified method of D. Amour and Smith[8] called as tail flick method using an analgesiometer. The tail flick method was commonly used to measure the analgesic activity. The tail flick method was utilized to study the analgesic activity in rats. A radiant heat automatic tail flick analgesiometer was used to measure reaction latencies. (Using tail flick analgesiometer type 812, UGO BASILE®, Germany). Animals were divided into four groups of 5 ratseach. Group, I served as normal control and received the vehicle (CMC 0.5% w/v in normal saline), where group IIwas injected by Pentazocin 30 mg/kg, i.p to act as a positive control. Group III and IV received SCM at doses 100 mg/kg and 200 mg/kg respectively. All drugs were injected intraperitoneally (i.p)30 min prior to the experiment. The pain was induced by giving radiant heat on the tail of the mice 5 cm away from the tip of the tail. Mice were held loosely during the test.Percentage of analgesia was calculated using the following formula:

% Analgesia = MPE=TL-BL / ML-BL × 100

Where M.P.E. = Maximum possible effect, M.L. = Maximum latency or cut off time

T.L. = Test latency, B.L. = Basal latency or control latency

5 rats in each group which were as follows:

Group I: Normal control (CMC 0.5% w/v in normal saline)

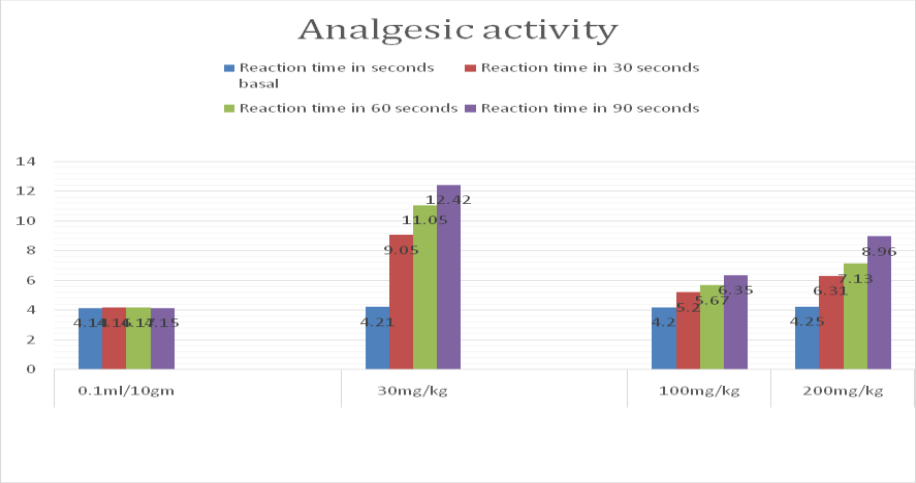
Group II: Pentazocin (30 mg/kg, i.p)

Group III: Test 1(100 mg/kg)

Group IV: Test 1(200 mg/kg)

Reaction time was recorded as the interval time of exposing the tail to the light beam and the withdrawal of the tail. The latency period for responses was noted at various time intervals. The basal reaction time of animals to radiant heat was recorded by locating the tipm(Last 1-2 cm) of the tail on the radiant heat source. The tail removal from the radiant warmth was taken as an endpoint. The cutoff time of 15 seconds was used to avoid tail injury by radiation[9]. The latent period of the tail-flick response was determined at 30, 60 and 90 minutes after the administration of standard and test.

RESULTS



Graph 3: Showing Analgesic Activity.

The effect of Strychnos colubrina methanolic (SCM) leaf extracts on blood Glucoselevel

Administration of streptozotocin produced 229.6% increase in fasting blood glucose levels of diabetic control rats compared to the normal control rats and the increased glucose levels in group I rats was maintained over a period of four weeks. Administration of *Strychnos colubrina* methanolic (SCM) leaf extracts to streptozotocin-induced diabetic rats for four weeks produced a significant blood glucose reduction (Table 1). Change orreduction in blood glucose level was observed from the first week by both SCM extract and glibenclamide. At the end of 4th week, 100 mg/kg b.w. of extract produced 27.2% blood glucose reduction in group IV rats. Similar to group IV rats, there was a lowering of 29.2% blood glucose in the rats treated with 200 mg/kg b.w. of the extract. Among the two doses of the extracts used, 200 mg/kg b.w. of the extract showed a greater reduction in blood glucose level which was comparable to glibenclamide.

The diuretic effect of Strychnos colubrina methanolic (SCM) leaf extracts

In this model when compared to control group the *Strychnos colubrina* methanolic (SCM) leaf extractstreated groups at different dose levels (100and 200 mg/kg) was noted with a great increase in the urine volume and also profoundly increased the excretion of ions like Sodium, Potassium and Chloride in urine.100 mg/kg b.w. of extract produced 81.4% of total urine output, 92.4% sodium ions, 91.2% potassium ions and 59.3% chloride ions excreted in group III rats. Similarly in group IV rats, there was 89.9% of urine output,94.4% sodium ions, 95.3% potassium ions and 61.23% chloride ions excreted in the rats treated with 200 mg/kg b.w. of the extract. Among the two doses of the extracts used, 200 mg/kg b.w. of the extract showed a greater diuretic effect by pumping out more urine and ions which was comparable toFurosemide (standard).

Analgesic effect of Strychnos colubrina methanolic (SCM) leaf extracts

In tail flick test, SCM in a concentration-dependent manner, exhibited significant analgesic activity by increasing the latency time of responses in rats in the two tested doses 100mg/kg and 200 mg/kg, as can be seen in Table 3. In comparison to standard drug (Pentazocin)100 mg /kg of SCM produced 30% and 200 mg/kg of SCM produced 66% analgesic activity in tail flick examination. Between two tested samples, 200 mg/kg has shown more analgesic activity than the former one.Maximum possible effects of drugs in tail flick model of analgesia in rats is shown in table 4.

DISCUSSION

The effect of two different doses of *Strychnos colubrine* methanolic (SCM) leaf extracts on the fasting blood glucose levels of diabetic rats is given in Table 1 and graph 1. In the diabetic rats, the methanolic extract at a dose of 200 mg/kg b.w. produced a 29.2% fall in the blood glucose levels at the end of the experimental period. The glibenclamide treated diabetic rats showed a greater reduction in the blood glucose level than the extract treated rats. Even though there was a reduction in blood glucose levels with 200 mg/kg b.w. of the extract, the decrease was much lower when compared to group III (glibenclamide) animals.

Analgesics are drugs that act on the central or peripheral nervous system to relieve pain selectively without altering consciousness[10]. Basically analgesics act by increasing the threshold for pain and changing the physiological response to pain.The analgesic activity of SCM was studied by the tail flick tests, which is a standard pharmacological model for the analysis of pain by natural products[11]. Tail flick method is used generally for centrally acting analgesics[12]. Results have shown that a single dose administration of standard Furosemide and *Strychnos colubrina* methanolic (SCM) leaf extracts significantly increased the urine output along with an increase in the elimination of Sodium, Potassium, and Chloride ions. *Strychnos colubrina* methanolic (SCM) leaf extracts of 200 mg/Kg produced a comparable diuretic activity with standard Furosemide.

CONCLUSION

The present study demonstrates that *Strychnos colubrina* methanolic (SCM) leaf extracts act as a potent antidiabetic, diuretic, and analgesic agent. The analgesic activity may be due to its ability to activate opioid receptors in the central nervous system. It may also inhibit endogenous pain substances, which are involved in the peripheral analgesia. The results obtained here with support the folkloric claims regarding the plant and its medicinal values. This paper helps in formulating natural principles to combat drug resistance of certain. It is, therefore, implied the isolation and proper characterization of the active constituents from the extracts of this plant species as possible antidiabetic, diuretic and analgesic agent.Quantitative analysis of the active compound in *Strychnos colubrina L.* leaf extracts should be further studied in the nearest future. Hencethis report provides insight into the medical potential of the phytomedicine and for further research on these drugs.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interests.

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