Acute toxicity test and Antihyperglycemic activity of the Leaves of *Senna auriculata* (L.) Roxb. (Peik-thin-gat)

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ABSTRACT

The plant *Senna auriculata* (L.) Roxb., belongs to the family Fabaceae. It is a potential folklore medicinal plant, which is widely used to treat diabetes mellitus. *Senna auriculata* (L.) Roxb. leaves extract was investigated to reveal its antihyperglycemic effect and acute toxicity study. The powdered leaves were tested for the phytochemical constituents. Alkaloid, glycoside, carbohydrate, α-amino acid, phenolic compound, flavonoid, terpenoid, steroid, tannin and reducing sugar were present. The acute toxicity study of 70% ethanolic extract was carried out according to the OECD test guideline 423. There were no signs of toxicity of mice even with a maximum dose of 5g/kg of the extract during 14 days. For evaluation of antihyperglycemic activity, 70% ethanolic extract were administered by oral route to adrenaline induced hyperglycemic rat model. The dose of 4g/kg showed significant blood glucose lowering effect at 2 hours ($P < 0.05$), 3 hours ($P<0.01$) and 4 hours ($P < 0.001$) when compared with the control. *Senna auriculata* (L.) Roxb. leaves possesses significant antihyperglycemic activity in adrenaline induced rat model and it is practically non-toxic.

Keywords: *Senna auriculata* (L.) Roxb., Phytochemical Test, Acute toxicity study, and Antihyperglycemic Activity.

Introduction

Senna auriculata (L.) Roxb. is a medicinal plant, belongs to family Fabaceae, which grows abundantly in Asia.It is widely used in Ayurvedic medicine as tonic, astringent and as remedy for diabetes, conjunctives and opthalmia (Kalaivani *et al*., 2008).

The medicinal value of this plant lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds.Pai Aruna and Karki Roopa. 2011., revealed that the extracts of *Senna auriculata* plants possess significant antidiabetic activity in alloxan- induced rats.

As per OECD guidelines, toxicological studies are very essential in order to establish the safety and efficiency of a new drug prior to clinical use. Acute toxicity tests are commonly used to determine LD_{50} of drugs and natural products (Mir *et al*., 2013).

Diabetes mellitus is basically a metabolic disorder associated with excess accumulation of glucose in blood. The two main forms of diabetes are type 1 or insulin dependent diabetes mellitus (IDDM) and type 2 diabetes or non-insulin-dependent diabetes mellitus (NIDDM). (Rani *et al*.2014).

Many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic presentation including *Senna auriculata* (L.) Roxb. The present investigation was designed to evaluate the acute toxicity effect and antihyperglycemic properties of *Senna auriculata* (L.) Roxb. leaves.

Materials and Methods

The plant materials were collected from Nyaung Oo Township, Mandalay Region, during the flowering period from September to February, 2016. The collected specimens were photographed to record the data and identified by using available literatures; Kurz (1877), Hooker (1879), Kirtikar and Basu (1933), Burkill (1935),

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Backer (1963), Lawrance (1964), Purseglove (1969), Dassanayake (1991). The collected leave specimens were washed thoroughly and air-dried in room temperature for two weeks. After that, the dried samples were pulverized by grinding to get powder and stored in air tight containers.

Preliminary Phytochemical investigation of *Senna auriculata* (L.) Roxb.

The preliminary phytochemical investigation has been undertaken on the leave of *Senna auriculata* (L.) Roxb**.,** to determine the presence or absence of organic constituents. The tests were carried out according to the standard method of British Pharmacopoeia (1968), Central Council for Research in Unani Medicine (1987), Marini Bettolo *et al*. (1981), Harbone (1984) and Trease and Evans (2002). Preliminary phytochemical examination was carried out at the Pharmaceutical and Research Department (PRD).The results were shown in Table (1).

Acute toxicity study of 70% ethanolic extract from leaves

(1) Materials

Test animals – Female albino mice (Dutch Denken Yoken Strain, body weight 25-30 g)

Test agents – 70% ethanolic extract from leaves of *Sennaauriculata* (L.) Roxb.

Apparatus – Animal balance, Mice cages, 18gauge intragastric needle

Dose schedule – 300mg/kg, 2000mg/kg and 5000mg/kg (body weight) of mice.

(2) Method

Acute toxicity test was performed according to OECD guideline TG 423 (2001) in order to estimate LD_{50} value of ethanolic extract. The range of acute toxicity of the test substance depends on the mortality and moribund status of the animals. Experiments were performed using healthy young adult female albino mice, nulliparous, non-pregnant, ddy strain and weighing 25-30 g. The test was carried out in Animal Service Division, and Pharmacology Research Division, DMR.

The animals were randomly divided into three groups containing six mice but only two in 5g/kg group. They were identified by the markings using a yellow stain. The test substances were administered in a single dose by gavage using mice intragastric needle. Animals were fasted 4 hours prior to dosing (only food was withheld for 4 hours but not water).

Following the period of fasting, animals were weighed and test substance was administered orally. Six animals (three animals per step) and two animals were used for each dose level. Starting dose of the sample, 300 mg/kg, body weight was used. So, another 8 mice were administered 2000 mg/kg and 5000 mg/kg body weight.

Animals were observed individually after dosing at least once during the first 30 minute, periodically during the first 24 hour, with special attention was given during the first 4 hours, and daily thereafter, for a total of 14 days.

All observations were systematically recorded with individual record being maintained for each animal. Signs of toxicity and mortality of the animal were recorded.

Individual weight of animals was measured shortly before the test substance administered and once weekly. Weight changes were calculated and recorded.

Determination of antihyperglycemic activity of 70% ethanolic extract from leaves in adrenaline induced hyperglycemic rats

(1) Materials

Test animals – Male albino rats (Wistar Strain, 250-300g).

Drugs – Adrenaline tartarate (1mg/ml, Myanmar Pharmaceutical factory), Glibenclamide (Hovid,

Malaysia), Betadine and 70% ethanolic extract from leaves of *Sennaauriculata* (L.) Roxb.

Instruments and Apparatus – Glucose Test meter (GT-1640 Arkray Co. Ltd; Japan), test strips (Glucocard™ Test strip II, Arkray Co. Ltd; Japan), 18gauge intragastric needle, Disposable syringes with needle (1ml, 5ml), Sprit cotton wool, Rat cages, Animal balance and Mechanical restraint devices.

(2) Methods

The study of antihyperglycemic effect of 70% ethanolic extract of *Senna auriculata* (L.) Roxb. leaves were performed by using the method of (Gupta et al.,1967 and Agrawl and Paridhavi,2007). Seven adult healthy Wistar Strain albino rats, weighing between 250-300g were used for the study. The test was carried out in the Department of Medical Research at Animal Service Division and Pharmacology Research Division. The animals were kept in clean and dry cages to allow for acclimatization to the laboratory conditions one week before starting. Before the experiment, the animals were weighed and marked to permit individual identification and kept fasting overnight for 18 hours but were allowed with free access to water. The dosage of the extract was calculated on the body weight basis for each rats.Blood samples were collected and baseline fasting blood sugar levels (0 hour) were measured by cutting about 0.4 cm of tip of the tail.The different doses of 10 ml/kg of distilled water (control), 70% ethanolic extract (1 g/kg, 2 g/kg, 4 g/kg) and standard drug (glibenclamide, 4 mg/kg) were administered orally to rats.Test extract, distilled water or standard drug were given by using an intragastric needle connected to a plastic syringe containing the calculated dosages.The needle was put into the mouth about 5 cm and then, the piston was pushed to deliver the test substances into stomach until they reached the stomach.After 30 minutes, these rats were induced hyperglycemia by giving adrenaline tartarate 0.4 ml/kg subcutaneously to nape of neck of the rats.Then further samples of blood were collected by squeezing the tip of tail which had been cut at 1hr, 2 hours, 3 hours and 4 hours after giving the dosage.Blood glucose was detected with glucometer and the results were recorded. The procedure was shown in figure(1-3)

Principle of blood glucose concentration

The blood sample is drawn into the test strip through capillary action. Glucose in the sample reacts with glucose oxidase and potassium ferricyanide in the strip, producing potassium ferrocyanide in proportion to the glucose concentration of the blood sample. Oxidation of the potassium ferrocyanide produces an electrical current which is then converted by the meter to display the glucose concentration. The detection method is biosensor technology.

Data management and analysis

Standard statistical methods were used in the calculation of arithmetic mean ($\bar{X}$), Standard Deviation (SD) and Standard Error (SE). Unpaired student "t" test was used to observe the significance of difference between means of both control and experimental groups. Changes were considered significant if the P-value was $P< 0.05$.

Fig. (1) Measuring of blood glucose concentration with glucometer

Fig. (2)Subcutaneous injection of adrenaline

Fig. (3) Administration of extract suspension

RESULTS

Morphological characters of *Senna auriculata* (L.)Roxb.

Perennial shrubs up to1.5-2.5 m high. Leaves are alternate, unipinnate and paripinnately compound; petioles stout, cylindrical, slightly canaliculate above; the racheae slightly canaliculated above, filiform glands present in between each pair of the leaflets; leaflets 8-12 pairs, oblong to elliptic- oblong , mucronate; petiolules, dark brown, tomentose; stipules auriculate or lunate-reniform with pointed appendages curved towards the leaves, green, persistent. Inflorescences terminal or axillary, corymbose racemes, 5 to 10 flowers, bracts ovate-acuminate, pedicels, pubescent; bracteoles linear, green, caducous. Flowers bright yellow, bisexual, zygomorphic, 5 merous, cyclic, hypogynous. Calyx; sepals-5, aposepalous, imbricate,brownish-yellow, coriaceous, glabrous, persistent, inferior. Corolla; petals 5, apopetalous, rosaceous, valvate, bright yellow, veins reddish brown, reticulate, glabrous. Stamens 10, apostamenous, 7 fertile and 3sterile, the fertile stamens 3 long and 4 short,the 3 sterile filaments anther lobes rounded, light brown, glabrous, inferior. Pistil monocarpellary, unilocular, one ovule in each locule, marginal placentation , ovary superior, tomentose; the styles slender, curved, glabrous; the stigma filiform, pubescent; the gynophores present. The pods dehiscent, oblongoid, flattened, the tips mucronate, dark green, flexible, glabrous. Seeds 10-20, ellipsoid, brown to dark brown, hard and glabrous.

Fig. (4) Habit of flowering plant

Fig. (5) Leaves

Fig. (6) Inflorescence

Fig. (7) Flower

Preliminary phytochemical investigation of *Senna auriculata* (L.) Roxb.

In preliminary phytochemical investigation, alkaloids, glycosides, reducing sugars, α-amino acids, carbohydrate, tannin, steroid, terpenoid, flavonoid, phenolic compound were observed in leaves. The tests have shown that cyanogenic glycoside, saponin and starch were absent. The results were shown in Table 1.

Table 1. Preliminary phytochemical investigation on leaves of *Senna auriculata* (L.) Roxb.

No.	Test	Extract	Test reagent	Observation	Results
1	Alkaloid	1 % HCl	1. Mayer's reagent 2. Dragendorff's reagent 3. Wagner's reagent 4. Hager's reagent	White ppt. Yellowish brown ppt. Deep blue ppt. Yellow ppt.	+ + + +
2	Glycoside	H_2O	10 % Lead acetate solution	White ppt.	+
3	Cyanogenic glycoside	H_2O	1. H_2O, Conc; H_2SO_4 2. Sodium picrate paper	No colour change	-
4	Saponin glycoside	H_2O	Distilled water	No persistent foam	-
5.	Starch	H_2O	Iodine solution	Brown ppt.	-
6	α-amino acid	H_2O	Ninhydrin reagent	Purple colour	+
7	Carbohydrate	H_2O	1. 10 % α naphthol , Conc: H_2SO_4	Red ring	+
8	Reducing sugar	H_2O	1. Fehling's solution	Brick red ppt.	+ +
9	Tannin	H_2O	1% gelatin & 10% NaClsolution	White ppt.	+
10	Phenolic Compound	H_2O	5% $FeCl_3$	Brownish green colour	+
11	Flavonoid	MeOH	Conc: HCl/ Mg ribbon	Pink colour	+
12	Steroid	PE	Acetic anhydride and conc: H_2SO_4	Bluish green colour	+
13	Terpenoid	$CHCl_3$	Acetic anhydrite and Conc: H_2SO_4	Pink colour	+

(+) = present, (-) = absent, ppt = precipitated

Acute toxicity test of 70% ethanolic extract from leaves of *Senna auriculata* (L.) Roxb.

The acute toxicity test for estimation of LD_{50} of extract was done according to the method described by OECD 423 (2001). In this experiment, there were no toxic signs or lethality during the observation period of 14 days with 300 mg/kg, 2000 mg/kg and the maximum dose of 5000 mg/kg. Therefore, the medium lethal dose (LD_{50}) of the extract was expected to be greater than 5000 mg/kg. All the animals did not display any signs of toxicity in observation period. Skin, fur, eyes, mucous, membrane, respiratory rate, motor activity and behavioral pattern were found to be normal. At the end of 14 days, there were no significant difference between body weights of the animals of test groups. The results were shown in Table (2).

Table (2) Acute toxicity tests of 70% ethanolic extract from leaves

No. of Group	Type of extract	No. of mice tested	Dosage	Observed period	No. of death
I	70% ethanolic extract	6	300 mg/kg	14 days	0/6
II	70% ethanolic extract	6	2000 mg/kg	14 days	0/6
III	70% ethanolic extract	2	5000 mg/kg	14 days	0/2

Antihyperglycemic Activity of 70% ethanolic extract of *Senna auriculata* (L.) Roxb. leaves in the adrenaline - induced hyperglycemic rats

The antihyperglycemic activity of 70% ethanolic extract of *Senna auriculata* (L.) Roxb. leaves were tested by using adrenaline - induced hyperglycemic albino rats. Male albino rats (250-300 g) were used in the study. Seven albino rats were orally administered with 70% ethanolic extract (1 g/kg, 2 g/kg and 4 g/kg) and glibenclamide (4 mg/kg).

Effect of subcutaneous adrenaline injection on blood glucose levels of the albino rats

The results of mean blood glucose concentrations of 7 albino rats treated with 10 ml/kg of distilled water (i.e. control group) at 0 hour, 1 hour, 2 hours, 3 hours and 4 hours after subcutaneous injection of adrenaline tartrate (0.4 ml/kg) were found to be 62.00 ± 1.84 mg/dl, 182.8 ± 8.31 mg/dl, 243.57 ± 19.08 mg/dl, 207.00 ± 14.67 mg/dl and 171.00 ± 9.57 mg/dl respectively. It was found that significant hyperglycemia started at 1 hour and lasted up to 4 hours.

Antihyperglycemic effect of different doses of 70% ethanolic extract from leaves of *Senna auriculata* (L.) Roxb. in adrenaline- induced hyperglycemic rats

The results of mean blood glucose concentration of 7 albino rats treated with 70% ethanolic extract from leaves of *Senna auriculata* (L.) Roxb. (1 g/kg) at 0 hour, 1 hour, 2hours, 3 hours and 4 hours after subcutaneous injection of adrenaline (0.4 ml/kg) were 67.00 ± 1.50 mg/dl, 184.57 ± 7.16 mg/dl, 249.14 ± 10.58 mg/dl, 195.29 ± 6.49 mg/dl and 155.71 ± 6.53 mg/dl respectively.

The results of mean blood glucose concentrations of 7 albino rats treated with 70% ethanolic extract from leaves of *Senna auriculata* (L.) Roxb. (2 g/kg) at 0 hour, 1 hour, 2 hours, 3 hours and 4 hours after subcutaneous injection of adrenaline tartrate (0.4 ml/kg) were 60.43 ± 1.49 mg/dl, 169.14 ± 7.14 mg/dl, 204.71 ± 5.87 mg/dl, 181 ± 7.74 mg/dl and 138.86 ± 3.59 mg/dl respectively. Significant decrease in blood glucose level was found at 4 hr ($p < 0.05$) when compared with that of control.

The results of mean blood glucose concentration of 7 albino rats treated with 70% ethanolic extract of leaves of *Senna auriculata*(L.) Roxb. (4 g/kg) at 0 hour, 1 hour, 2 hours, 3 hours and 4 hours after subcutaneous injection of adrenaline tartrate (0.4 ml/kg) were 71.86 ± 1.77 mg/dl, 162.86 ± 2.91 mg/dl, 194.29 ± 3.99 mg/dl, 158.29 ± 2.90 mg/dl and 101.43 ± 3.52 mg/dl respectively. Significant decrease in blood glucose levels were found at 2 hours ($p < 0.05$), 3 hours ($p < 0.01$) and 4 hours ($p < 0.001$) when compared with that of control as shown in Table (3.12) and Figure (3.26). Mean blood glucose concentration of 70% ethanolic extract (1 g/kg, 2 g/kg and 4 g/kg) and glibenclamide (4 mg/kg).

Effect of standard drug glibenclamide (4 mg/kg) on blood glucose levels in adrenaline- induced hyperglycemic rats

The mean blood glucose levels of the 7 albino rats treated with standard drug (glibenclamide, 4 mg/kg) at 0 hour, 1 hour, 2 hours, 3 hours and 4 hours after subcutaneous injection of adrenaline (0.4 mg/kg) were 70.86 ± 2.05 mg/dl, 138.43 ± 6.34 mg/dl, 155.71 ± 4.31 mg/dl, 118.14 ± 3.47 mg/dl and 82.29 ± 4.27 mg/dl respectively.Significant reduction in blood glucoselevels was found at 2 hour ($p < 0.01$), 3 hours ($p < 0.01$) and 4 hours ($p < 0.001$) when compared with control group.

In comparison of antihyperglycemic effect of 70% ethanolic extract (4 g/kg) and standard drug (glibenclamide, 4 mg/kg), the antihyperglycemic effect of standard drug (glibenclamide) was found to be more than that of 70% ethanolic extract of leaves. The result was shown in Figure (8).

Mean percent inhibition of hyperglycemia with different doses of 70% ethanolic extract of the leaves of *Senna auriculata* (L.) Roxb.and standard drug (glibenclamide).

The mean percent reduction of hyperglycemia with 70% ethanolic extract (1 g/kg) were 1.23%, -3.90%, 8.40% and 16.83% at 1 hr, 2 hrs, 3 hrs and 4 hrs respectively.The mean percent reduction of hyperglycemia with 70% ethanolic extract (2 g/kg) were 8.93%, 17.37%, 14.23% and 26.38% at 1 hr, 2 hrs, 3 hrs and 4 hrs respectively.The mean percent reduction of hyperglycemia with 70% ethanolic extract (4 g/kg) were 21.86%, 21.68%, 34.52% and 69.75% at 1 hr, 2 hrs, 3 hrs and 4 hrs respectively.The mean percent reduction of standard drug (glibenclamide, 4 mg/kg) were 43.85%, 51.13%, 64.93% and 87.95% at 1 hr, 2 hrs, 3 hrs and 4 hrs respectively. The results were shown in Table (4) and Figure (15).As a comparison of antihyperglycemic effect of different doses of 70% ethanolic extract (1 g/kg, 2 g/kg and 4g/kg) of the leaves of *Senna auriculate* (L.) Roxb., the antihyperglycemic effect showed dependent on the doses of the extract.The result was shown in Figure (9).

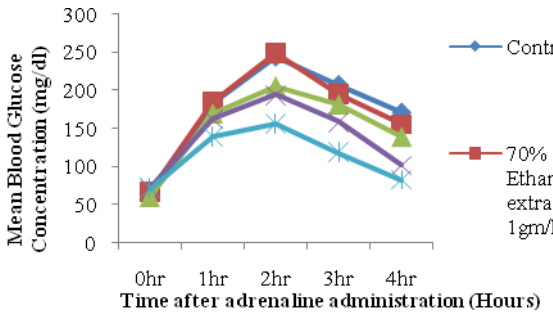

Fig. (8) Time course effect of 70% ethanolic extract of *Senna auriculata* (L.) Roxb. leaves (1 g/kg, 2 g/kg and 4 g/kg) and standard drug (glibenclamide, 4 mg/kg) on adrenaline induced hyperglycemic rat model

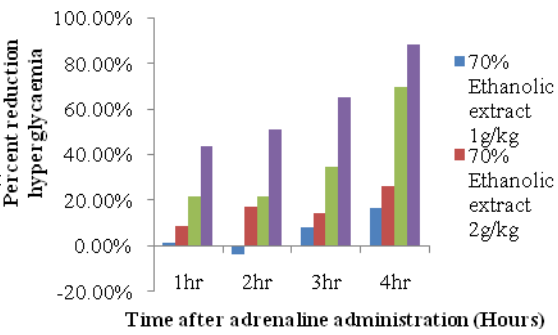

Fig. (9) Mean percent reductions of hyperglycemia with different dose levels of *Senna auriculata* (L.) Roxb. leaves (70% ethanolic extract) and standard drug (glibenclamide) on adrenaline induced hyperglycemic rat model. Sample size for each group (n=7)

Discussion and Conclusion

In this research, the morphological studies are focused on both vegetative and reproductive parts of the plants.In morphological studies, *Senna auriculata* (L.) Roxb. are perennial shrubs. Leaves are paripinnately compound, filiform glands present and each gland between each pair of leaflets. Stipules auriculate or lunate-reniform. Inflorescences are terminal or axillary corymbose raceme.

Flowers are bright-yellow, bisexual and hypogynous.The fruits are dehiscent pod. The seeds are ellipsoid, brown or dark brown, glabrous. These characters are in agreement with those described by Kurz, 1877; Hooker, 1879; Kirtikar and Basu, 1933, Burkill, 1935; Cooke, 1958; Backer, 1963; Purseglove, 1969; Dassanayake, 1991.

In this paper, the preliminary phytochemical tests, Acute toxicity study and antihyperglycemic activity of *Senna auriculata* (L.) Roxb. have been described. According to phytochemical tests, the results revealed that alkaloids, glycosides, phenolic compounds, reducing sugar, carbohydrates, tannins, flavonoids, terpenoids, steroids and α- amino acids were distinctly found and starch, cyanogenic glycosides and saponins were absent.

The acute toxicity study of 70% ethanolic extract of *Senna auriculata* (L.) Roxb. leaves revealed no mortality when administered orally up to a maximum dose of 5 g/kg body weight during the period of 14 days. At this dose there was no gross behavioral changes. So, LD_{50} cut off value of the extract was expected to more than 5 g/kg (body weight).The acute toxicity test experiment was coincided with Sharma *et al.*, 2009 and Mhetre, N. K. *et al*., 2014.

In this experiment, different doses of 70% ethanolic extract (2g/ kg and 4g/ kg) had shown significant decreases in the blood glucose concentration at 2 hours ($P<0.05$), 3 hours ($P<0.01$), 4 hours ($P<0.05$, and $P< 0.001$) when compared with that of control. The percent reduction of hyperglycemia with 70% ethanolic extract (4g/ kg) at 1 hour, 2 hours, 3 hours and 4 hours were 21.86%, 21.68%., 34.52% and 69.75% respectively.

In the present study, glibenclamide was used as the standard drug at the dose level of 4mg/kg and it showed a significant antihyperglycemic effect, started from

|1 hour up to 4 hours ($P<0.05$ and $P<0.01$) after oral administration of the drug to the albino rats with a maximal activity at 4 hours ($P< 0.001$). Percent reduction of hyperglycemia by glibenclamide at 1 hour, 2 hours, 3 hours and 4 hours were 43.85%, 51.13%., 64.93% and 87.95% respectively.

As a comparison of antihyperglycemic effect between glibenclamide (4 mg/kg) and different doses of 70% ethanolic extract of the leaves of *Senna auriculata* (L.) Roxb. (1g/ kg, 2g/ kg and 4g/ kg), it was found that the glucose lowering effect of 70% ethanolic extract of the leaves of *Senna auriculata* (L.) Roxb. was less than that of glibenclamide.The result of the study proved that the *Senna auriculata* (L.) Roxb. leaves possess significant antihyperglycemic activity along with potent antioxidant potential in diabetic conditions.

It is concluded that, the ethanol extracts of *Senna auriculata* (L.) Roxb. leaves might be a potential alternative agent for antihyperglycemic activity. It may be due to abundant presence of flavonoid and phenolic compounds. This investigation may focus on research fields to develop clinical studies which might be of great scientific contribution for the society.

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