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Hypoglycemic and antihyperglycemic activity of leaf extract of *pluchea indica* Less.

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SUMMARY

The hypoglycemic and antihyperglycemic activity of methanolic extract of *Pluchea indica* Less. (Asteraceae) (MEPI) leaves were studied in normal rats and in streptozotocin induced diabetic rats respectively. The blood glucose levels were measured at 1, 4, 8, 16 and 24 h intervals after the treatment. The MEPI leaves showed reduction in blood glucose level in normal (35.12% and 36.01% for 200 and 400 mg/kg, p.o. respectively) and in steptozotocin induced diabetic rats (36.10% and 41.87% for 200 and 400 mg/kg respectively). A toxicity study has been performed for the extract, which revealed that the extract is safe to use even at the doses of 3.2 gm/kg of body weight orally.

Key words: *Pluchea indica*; Leaves; Hypoglycemic; Antihyperglycemic; Streptozotocin

INTRODUCTION

The plant *Pluchea indica* Less. (Family: Asteraceae) is an evergreen large shrub found abundantly in salt marshes and mangrove swamps in Sunderbans. In Indo-China the roots in decoction are prescribed in fevers as a diaphoretic and an infusion of the leaves is given internally in lumbago. The root and leaves are used in Patna as astringent and antipyretics (Kirtikar and Basu, 1999). The plant is also known to be used in rheumatoid arthritis (Chatterjee, 1996). The root extract has also been evaluated to possess anti-inflammatory (Sen *et al.*, 1991) and antiulcer (Sen *et al.*, 1993) activities. The plant has also been reported to possess diuretic (Muangman *et al.*, 1998) effects. Therefore a thorough chemical investigation was made by us to elucidate the active constituents from the roots of the plant *Pluchea indica*, which is already known for its multifarious activities. So far a number of chemical constituents have been isolated from different parts of the plant. A new eudesmane derivative from the leaves (Mukhopadhyay *et al.*, 1983), five new terpenic glycosides from aerial parts (Uchiyama *et al.*, 1989), three new eudesmane-type sesquiterpenes and three new lignan glycosides, together with a known eudesmane-type sesquiterpene from roots (Uchiyama *et al.*, 1991) and two new thiophene derivatives, besides two pentacyclic triterpenes of rare occurrence from roots (Chakravarty *et al.*, 1994) have been isolated from this plant.

The hypoglycemic effect of roots of *Pluchea indica*

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Less. is already reported (Peungvicha *et al.*, 1999). As the use of the leaves of the plant for diabetes is not supported by experimental and clinical data in any literature, but it is widely used by the Lepcha people of Darjeeling, so we attempt to evaluate the hypoglycemic and antihyperglycemic activity of methanol extract of the leaves of *Pluchea indica* Less. (MEPI) in normal and in streptozotocin induced hyperglycemic rats.

MATERIALS AND METHODS

Plant material

The plant *Pluchea indica* Less. (Family: Asteraceae) was collected in the month of July 2004 from the region of Sunderbans, West Bengal, India. The plant was taxonomically identified and authenticated by Botanical Survey of India, Sibpur, India. The leaves of *Pluchea indica* were separated, washed, oven-dried at 60°C, powdered and sieved through 100 mesh. Fibres and unwanted materials were rejected after sieving. The powder was preserved in an airtight container for further use.

Preparation of the extract

The powdered leaves (500 g) were extracted in petroleum ether under room temperature. The solvent was filtered out and the dried powder was extracted in a Soxhlet extraction apparatus with methanol for 20 h. The methanol was evaporated under reduced pressure by rotary vacuum evaporator and a sticky dark brown residue (75 g) was obtained (Yield, 15%). This was stored in a desiccator and refrigerated at 4°C for future use. The methanolic extract was dissolved in 2% v/v aqueous Tween 80 solution prior to the experiment and administered orally (p.o.).

Animals

Swiss albino mice (20 - 22 g) and Wistar albino rats (180 - 200 g) of either sex were selected for the experiment. The animals were kept under standard conditions of 12 : 12 h light and dark cycle in polypropylene cages and fed with standard laboratory diet and water *ad libitum*.

Chemicals and reagents

Streptozotocin (Sigma, U.S.A.); Glucose assay kits (Span diagnostics, Surat, India). All the solvents used were of analytical grade.

Phytochemical studies

The preliminary phytochemical screening of the extract (MEPI) were done using standard procedures (Stahl, 1969) and confirmed by TLC revealed the presence of various types of chemical groups like glycosides, phenols, terpenoids, sterols, tannins and reducing sugars.

Toxicity studies

Acute toxicity relating to the determination of LD_{50} value was performed with different doses of the extract (Ghosh, 1994) that revealed that the extract is safe to use even at the doses of 3.2 g/kg of body weight orally.

Blood collection

Blood samples were drawn by retro-orbital puncture and plasma was separated within 30 min of the collection for determination of blood-glucose level.

Determination of blood glucose levels

The blood glucose level was determined by the ortho - toluidine method (Hutman *et al.*, 1959; Hyavariner *et al.*, 1962). 0.9 ml of trichloroacetic acid solution (3% w/v) was taken in a centrifuge tube and 0.1ml of blood sample was added. The content of the tube was mixed and then centrifuged for 10 min at 3,000 rpm. 0.5 ml of centrifuged was taken into a test tube was placed in a boiling water bath for 8 min and then it was allowed to cool under a stream of tap water. The absorbance was measured in a photocolorimeter at 620 nm.

Effect of MEPI on normal blood glucose level

Rats were divided into four groups (n = 6) and

fasted for 18 h before the experiment. First group received the control vehicle 2% v/v aq. Tween 80 solution (10 ml/kg, p.o.). The methanol extract of *Pluchea indica* Less. leaves (200 and 400 mg/kg, p.o.) were administered to second and third groups respectively. The fourth group received the standard drug glibenclamide (10 mg/kg, p.o.) for assessing the comparative pharmacological significance (Sharma *et al.*, 1997). The plasma glucose concentration was determined at 1, 4, 8, 16 and 24 h after MEPI administration.

Effect of MEPI on streptozotocin induced diabetic rats

The rats were made hyperglycemic by a single dose of intraperitoneal injection with streptozotocin (60 mg/kg, Sigma, U.S.A.). 48 h after the injection, blood glucose concentration in all the surviving rats were estimated. Rats that were exhibiting plasma glucose concentration of 250 - 350 mg/dl were considered to be diabetic and were used for the experiment. The rats were divided into four groups (n = 6), drugs were administered and the glucose levels were estimated in a similar manner.

Statistical analysis

All results are expressed as the mean ± S.E.M. The results were analyzed for statistical significance by one - way analysis of variance (ANOVA) followed by Dunnett's test using computerized Graph Pad InStat version 3.05, Graph pad software Inc., San Diego, U.S.A.

RESULTS

Assessment of acute toxicity

In the LD_{50} determination it has been observed that the extract is safe to use in animals even at a dose of 3.2 g/kg (Table 1).

Assessment of normal blood-glucose level

The hypoglycemic activity of the methanol extract of Pluchea indica was studied in normal rats for 24 h after oral treatment at the dose levels of 200 and 400 mg/kg. MEPI reduced the blood glucose level significantly ($P < 0.001$) from 4 h after oral administration, when compared with the control groups. It was also noted that the blood glucose-lowering efficacy persisted up to 24 h after treatment. The effect was comparable to that of the effect produced by the standard drug, glibenclamide 10 mg/kg (Table 2).

Assessment of streptozotocin-induced blood-glucose level

The extract exhibited a significant antihyperglycemic effect in the streptozotocin induced diabetic rats at 4 h after drug administration and the activity was also persisted upto 24 h. The extract at the dose of 400 mg/kg showed the maximum activity of 41.87 % ($P < 0.001$) reduction in the glucose level was observed at 24 h after drug administration when compared with that of control group. The effect was also comparable with that of effect produced by the standard oral hypoglycemic drug

Table 1. Toxicity study of MEPI in mice

Treatment	Dose(mg/kg, p.o.)	No. of animals	No. of survivals	No. of death	LD_{50}
Control	10 ml	20	20	0	
MEPI	100	20	20	0	
MEPI	200	20	20	0	
MEPI	400	20	20	0	
MEPI	800	20	20	0	
MEPI	1600	20	20	0	
MEPI	3200	20	20	0	> 3.2 g/kg

It has been found that the MEPI is safe to use in animals even at a dose of 3.2 g/kg (orally).

Table 2. Effect of MEPI on Normoglycemic rats

Treatment	Dose (mg/kg, p.o.)	Plasma glucose concentration (mg/dl) Time after treatment (h)				
		1 h	4 h	8 h	16 h	24 h
Control	10 ml	106.33 ± 3.7	112.33 ± 3.8	112.67 ± 3.6	112.83 ± 3.3	112.00 ± 3.0
MEPI	200	100.33 ± 3.7*** (5.64)	86.66 ± 4.4* (22.85)	83.00 ± 4.0* (26.33)	77.00 ± 4.3* (31.76)	72.67 ± 3.7* (35.12)
	400	97.17 ± 2.9** (8.61)	82.33 ± 2.6* (26.71)	80.83 ± 2.0* (28.26)	77.33 ± 2.7* (31.46)	71.67 ± 4.0* (36.01)
Glibenclamide	10	98.50 ± 3.9** (7.36)	89.50 ± 3.9* (20.32)	82.17 ± 2.6* (27.07)	80.33 ± 3.8* (28.80)	73.67 ± 4.0* (34.22)

*$P < 0.001$, **$P < 0.01$, *$P < 0.05$, vs. control. Values are expressed as mean ± S.E.M. (n = 6). The results were analyzed by ANOVA followed by Dunnett's test. Data in parenthesis indicate % of inhibition when compared to control.

Table 3. Effect of MEPI on streptozotocin induced diabetic rats

Treatment	Dose (mg/kg, p.o.)	Plasma glucose concentration (mg/dl) Time after treatment (h)				
		1 h	4 h	8 h	16 h	24 h
Control	10 ml	296.83 ± 5.4	295.67 ± 4.6	305.00 ± 3.7	305.33 ± 7.1	297.33 ± 2.8
MEPI	200	278.17 ± 4.3* (6.29)	247.83 ± 2.3* (16.18)	227.50 ± 4.5* (25.41)	210.33 ± 4.6* (31.11)	190.00 ± 4.6* (36.10)
	400	257.33 ± 5.2* (13.31)	220.17 ± 2.3* (25.54)	204.17 ± 4.0* (33.06)	185.00 ± 6.5* (39.41)	172.83 ± 3.7* (41.87)
Glibenclamide	10	277.67 ± 4.3* (6.45)	223.32 ± 4.3* (24.47)	198.17 ± 3.7* (35.03)	180.50 ± 4.2* (40.88)	168.00 ± 3.6* (43.50)

*$P < 0.001$, vs. control. Values are expressed as mean ± S.E.M. (n = 6). The results were analyzed by ANOVA followed by Dunnett's test. Data in parenthesis indicate % of inhibition when compared to control.

glibenclamide (10 mg/kg, 43.50%) (Table 3).

DISCUSSION

The extract showed significant hypoglycemic effect in normal and antihyperglycemic effect in streptozotocin induced diabetic rats in all the dose levels tested. The extract showed gradual onset and longer duration of action and was similar to that of standard oral hypoglycemic agent glibenclamide. MEPI reduced the plasma glucose level to 25.54% (400 mg/kg), which is more than the standard drug glibenclamide, which reduced only 24.47% (10 mg/kg) after 4 h in case of strepto-zotocin model. The antihyperglycemic activity in streptozotocin induced diabetic rats for extract is comparable (41.87% for 400 mg/kg) to that of the standard drug glibenclamide (43.50% for 10 mg/kg). It has been shown that the destruction of β- cells of the pancreas is directly proportional to the dose of the diabetogenic agent (Junod *et al.*, 1969). The present preliminary experimental results indicated that the drug exhibited a potent blood glucose lowering property both in normal and streptozotocin induced diabetic rats. Further studies like glycogen content of the different organs, cholesterol and triglyceride levels in blood, other enzymatic studies are to be performed to understand the exact mechanism of action, which are underway in our laboratory. Attempts have also been made to isolate the active constituents from the extract to understand the agents responsible for the above reported activity.

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