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A STUDY ON ASSEMENT OF DIURETIC ACTIVITY OF *BASELLA ALBA VAR RUBRA* IN WISTAR RATS AND MICE BY USING LIPSCHITZ MODEL

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ABSTRACT

The present study was carried out to evaluate the diuretic effect of ethanolic extract of *Basella alba extract (BAE)* in wistar rats and mice by using Lipschitz model respectively. *Basella alba extract (BAE)* showed significant ($p < 0.05$) diuretic and sedative activity of two doses (100mg/kg and 200mg/kg, p.o) tested, when compared to control and drugs furosemide and diazepam respectively. Hence, the *Basella alba extract (BAE)* possess diuretic activity.

KEYWORDS

Diuretic, Urine volume, Electrolytes, Fall of time and Furosemide etc.

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INTRODUCTON

The most widely used diuretics, the benzothiadiazine (e.g., chlorothiazide), interfere with the reabsorption of salt and water by the kidney tubules. After they were synthesized in the late 1950s, the benzothiadiazine replaced most other existing diuretics.

MATERIAL AND METHODS

Description

Its fruits are fleshy and stalk less, ovoid or spherical in shape, 5 to 6mm and purple when mature (Tropilab Inc. <http://tropilab.com/bas.html>. accessed on 31 July 2012). The useful parts of the plant include its leaves, young stem, matured fruit, and roots.

The stem of the *Basella alba* is green and the stem of the cultivar *Basella alba* 'Rubra' is reddish-purple; the leaves in both cases are green. *Basella alba* grows well under full sunlight in hot, humid climates and in areas lower than 500metres (1,600 ft) above sea level. The plant is native to tropical Asia⁵. It grows best in sandy loam soils rich in organic matter with pH ranging from 5.5 to 8.0^{44,45}.

Medicinal uses

Leaves of *Basella alba* is used for the treatment of hypertension and externally in treatment sores, urticaria and gonorrhoea. Antifungal, anticonvulsant, analgesic, anti-inflammatory and androgenic activities, demulcent, febrifuge, and laxative properties. It is beneficial to drink it during fluid retention, dysentery⁴⁶, diarrhoea, constipation and catarrh. Anti cancer, Anti viral, Anti bacterial, Anti microbial. Antioxidant, Anti depreesent, skeletal muscle relaxant. Diuretic⁴⁷, anti-ulcer activity. Anemia in women, anticancer such as melanoma, leukemia and oral cance⁴⁸.

Phytochemical investigation

Collection of plant material

Basella alba Linn var. (Fam. *Basellaceae*) leaves for the proposed study were collected from vegetable market of Guntur (India), fresh leaves of *Basella alba* with entire petiole were used in this study and this was authenticated and confirmed by botanist(P.SATYANARAYANA RAJU garu) at Acharya Nagarjuna University. The leaves after collection were washed to remove the debris and then shade dried and the dried leaves were powdered to get a coarse powder.

Preparation of Extract

The plant material was dried under shade and powdered mechanically. The 50gm of powder sample was defatted with petroleum ether (60-80°C) and then extracted with ethanol by using soxhlet apparatus. The extraction was continued till a few drops of the last portion of the extract left no residue on drying. The solvent was removed by concentrated in vacuo in a rotary evaporator and dried under reduced pressure. The yield of the methanol extract was 9.4%. The dried extract was stored in refrigerator. The extract were analyzed for the presence of alkaloids, carbohydrates,

saponins, tannins, flavonoids, phenols, steroidal glycosides, mucilage, anthocyanins.

In vivo studies

Animals used

Adult wistar albino male rats (150-180g) were procured from the laboratory animal model house are used in the study. The animals were kept under standard environmental conditions of room temperature ($22^{\circ}\pm 2^{\circ}\text{C}$), relative humidity ($50\%\pm 5\%$) and 12h light and dark cycle. The animals were housed in the colony cages (three rats per cage) and provided feed and water *ad libitum*. All the animals were acclimatized to the laboratory environment 5 days prior to experiment. The animal were fasted overnight just prior to the experiment but allowed free access to drinking water. All experiments were carried out in accordance with the guidelines of Institutional Animal Ethics Committee.

METHODS

Acute toxicity studies

Three wistar rats were selected for the study. The overnight fasted animals (with water *ad libitum*) were administered with ethanolic test extract at a single dose of 2000mg/kg body weight by orally. The dose volume was fixed at 10ml per kg body weight. The animals were observed for 0min, 30min, 1hr, 2hr, 4hr, 6hr, and there after every day 14 days. Food was with held for a further 3-4 hours after administration of test extract and was observed for signs for toxicity. The body weight of the rats before and after administration were noted that changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behaviour pattern was observed and also sign of tremors, convulsions, salivation, diarrhoea, lethargy, sleep, and coma was noted. At the end of 14th day the animals were sacrificed with excessive and dissected for examination of vital organs like brain, liver, kidney lungs, and heart for pathological changes. For further confirmation the above procedure was repeated on another set of 3 female wistar rats.

Evaluation of Diuretic Activity

Lipschitz method

For the assessment of Diuretic activity, the urine output and sodium, potassium and chloride levels in urine were measured. Here, the adult wistar rats were divided into 4 groups of six animals each. The were deprived of food and water for 16 hr prior to the experiment. Before the oral administration of test drug the animals are dosed with 25ml/kg body weight of normal saline. Among the four groups of animals, first group received normal saline (control) and the second group received the standard diuretic drug furosemide at 20mg/kg body weight. The extract was studied at two concentrations (100 and 200 mg/kg p.o) to the third and fourth groups respectively. Immediately after administration, the animals were placed in fabricated metabolic cages individually and urine was collected at 5hr and 24hr intervals. Total urine volume (ml/100gm), Na⁺, K⁺, and Cl⁻ concentrations in the urine was determined and diuretic index was calculated.

The treatment was as follows

Group I - control. (Normal saline 25ml/kg; p.o)

Group II - furosemide. (20mg/kg; p.o)

Group III - *Basella alba* (Test drug 100mg/kg; p.o)

Group IV - *Basella alba* (Test drug 200mg/kg; p.o)

Statistical Analysis

Result were analyzed for statistical by ANOVA followed by Dunnett's multiple comparison tests. Values $p < 0.05$ and below were considered significant.

RESULTS AND DISCUSSION

Phytochemical Studies

Plant material and extraction

Pressure The air dried and finely ground leaves of *Basella alba* was extracted by soxhlet apparatus with 95% ethanol at 40-50°C for eight hours, when filtered and concentrated under reduced gave the yield of 7.6% w/w. Hence forth this extract is called as *Basella alba* extract.

Preliminary phytochemical analysis

Preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates, saponins,

tannins, flavonoids, phenols, steroidal glycosides, mucilage, anthocyanin in *Basella alba* extract.

In –vivo studies

Acute toxicity studies

The Ethanolic extract of leaves of *Basella alba* (BAE) was found to be safe and no mortality of the rats was observed at the doses of 2000mg/kg for 14 days in acute toxicity study.

Diuretic activity

It was observed that BAE (*Basella alba* ethanolic leaves extract) has shown significant ($p < 0.05$) diuretic activity by increasing urine output and increased output of sodium, potassium, and chloride levels when compared to control. The effect of BAE (*Basella alba* extract) was found to be dose dependent i.e is among the two doses (100 and 200mg/kg; p.o) studied, higher dose (200mg/kg; p.o) produced more effect. A comparison was made with the standard furosemide. (Table No.3 and No.4, Graph No.1 and No.2).

Discussion

Preliminary phytochemical analysis revealed the presence of alkaloids, tannins and phenols flavonoids, and steroids in *Basella alba* leaves ethanolic extract.

In Acute toxicity study all the animals survived at the doses of 2000mg/kg body weight for a period of 14 days. So the LD50 of the BAE will be $> 2000\text{mg/kg}$ body weight.

The Diuretic effect of extract is indicated by increase in both water excretion and excretion of sodium, potassium, chloride ion. The BAE at 100mg/kg and 200mg/kg doses showed a statically significant dose dependent increase in the volume of urine and excretion of electrolytes hence BAE has Diuretic activity.

Table No.1: Percentage yield of Ethanolic extract of leaves of *Basella alba* extract

Weight of plant powder	250gm
Yield	19gms
Percentage yield	7.6%

Table No.2: Preliminary phytochemical analysis of ethanolic *Basella alba* (BAE)

S.No	Phytochemical Tests	Results
1	Test for alkaloids	+
2	Test for saponins	-
3	Test for tannins	-
4	Test for falvanoids	+
5	Test for carbohydrates	+
6	Test for steroidal glycosides	+
7	Test for anthocyanin	+
8	Test for mucilage	+
9	Test for phenols	-

+Ve: Indicates the presence of compounds

-Ve: Indicates the absence of compounds

Table No.3: Diuretic activity of BAE (*Basella alba* extract) On urine volume

Group	Treatment	Dose Mg/kg	Urine vol (ml) 5hrs	Diuretic index	Urine vol(ml) 24hrs	Diuretic index
1	Control	-	0.60	0.92	2.76	0.99
2	Furosemide	20	2.25 ± 0.83*	1.02	5.01 ± 0.47*	1.0
3	<i>Basella alba</i> extract (BAE)	100	1.30 ± 1.25*	1.39	3.76 ± 1.4*	1.23
4	<i>Basella alba</i> extract (BAE)	200	2.16 ± 0.75*	1.72	4.80 ± 0.36*	1.55

All values are mean ± SEM where (n=6) one way ANOVA followed by Dunnet's test * P < 0.05, ** P < 0.01 when compared to vehicle treated animals.

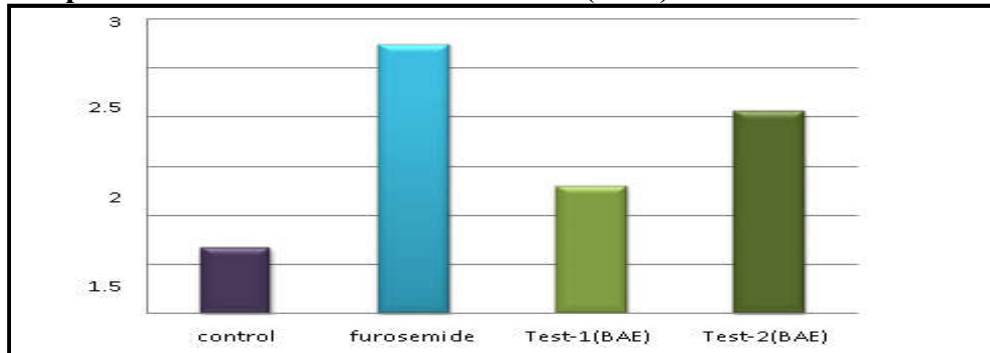
Diuretic activity of *Basella alba* extract BAE on electrolytes

Table No.4: Diuretic activity of *Basella alba* extract BAE on electrolytes

Group	Treatment	Dose mg/kg	Concentration of ions (mEq/L)			Saluretic index		
			Na ⁺	K ⁺	Cl ⁻	Na ⁺	Ka+	Cl ⁻
1	Control	-	125.3	3.34	84.5	1.02	1.01	1.00
2	furosemide	20	155.4 ± 3.1*	8.25±2.6*	136.5± 0.8*	1.19	2.35	1.56
3	<i>Basella alba</i> extract (BAE)	100	138.7 ± 2.4*	5.26±1.2*	102.7 ± 0.6*	1.02	1.46	1.20
4	<i>Basella alba</i> extract (BAE)	200	141.5±1.9 *	6.29 ± 1.3*	112.5±0.3*	1.08	1.81	1.35

All values are mean ± SEM where (n=6) one way ANOVA followed by Dunnet's test * P < 0.05, ** P < 0.01 when compared to vehicle treated animals.

Graph No.1: Effect of *Basella alba* extract (BAE) on urine volume at 5hrs



Graph No.2: Effect of *Basella alba* extract (BAE) on urine volume at 24hrs

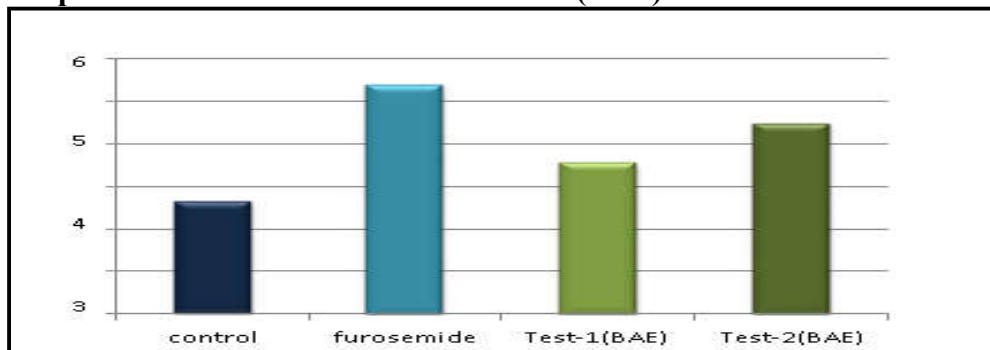


Figure No.1: *Basella alba*

CONCLUSION

The present study was aimed to assess the Diuretic and Sedative activity of Ethanolic *Basella alba* Leaf extract. The plant material was collected, Authenticated and extraction was done by using Soxhelt apparatus in three cycles with ethanol and finally powdered extract was prepared. Then the qualitative phytochemical analysis was done and observed the presence of alkaloids, tannins and phenols, steroidal glycosides, flavonoids and mucilage.

Toxicity studies were conducted in albino rats with Ethanolic extract of *Basella alba* leaves according to OECD guide line and was found safe up to the level of 2000mg/kg confirming its non toxic nature and no major behavioral changes were observed during the period of study. So for the present study two doses of 100mg/kg and 200mg/kg were taken. The Diuretic activity of the Ethanolic leaf extract of *Basella alba* was assessed by the method previously described by Lipschitz *et al.* The urine out and potassium, sodium, chloride levels in the urine were measured.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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