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Evaluation of Diuretic and Sedative Activity for Ethanolic Leaves Extract of Basella Alba L. Var Rubra

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ABSTRACT

Herbal medicine is the oldest form of health care known to mankind. Medicinal plants can be important sources of unknown chemica substances with potential therapeutic effects. The study of plant species with diuretic effects is still a fruitful research in search of nev diuretic and sedative drugs. The present study was carried out to evaluate the diuretic and sedative effect of ethanolic extract of *Basell alba (BAE)* in wistar rats & mice by using Lipschitz model and Rota rod model respectively. The urine volume, electrolyte levels in rat and fall of time of mices from rota rod were measured. BAE showed significant ($p<0.05$) diuretic and sedative activity of two dose (100mg/kg & 200mg/kg, p.o) tested, When compared to control and drugs furosemide and diazepam respectively. Hence, the BA possess diuretic and sedative activity.

Key words:
Diuretic, urine volume, electrolytes, sedative, fall of time, furosemide, diazepam.

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INTRODUCTION

Diuretics, in particular thiazide compounds, were the earliest forms of practical and well- tolerated antihypertensive drugs introduced in the modern therapeutic era. Numerous large randomized clinical trials have demonstrated the value of these agents in reducing morbidity and mortality in the hypertensive patient. Despite questions raised concerning the metabolic effects of thiazide diuretics, newer trials and guidelines have reaffirmed their primacy in the safe and 0.effective initial management of high BP and as an element of combination therapy in more complex or difficult cases. Contemporary strict BP treatment goals will require that diuretics continue to play a major role in hypertension therapy[1].

HISTORICAL BACKGROUND

Thiazide diuretics were introduced initially in 1957 and were quickly accepted as therapy for hypertension because they were effective, inexpensive, and well tolerated and because they could be used once daily in contrast to existing agents.1 Large randomized clinical trials in hypertensive patients subsequently proved their effectiveness in reducing morbidity and mortality. This chapter surveys early trial evidence, assesses the metabolic implications of diuretic use, and discusses recent trials and management guidelines that confirm the key role of diuretics in hypertensive patients[2].

DEFINITION

Diuretic, any drug that increases the flow of urine. Diuretics promote the removal from the body of excess water, salts, poisons, and accumulated metabolic products, such as urea. They serve to rid the body of excess fluid (edema) that accumulates in the tissues owing to various disease states. There are many types of diuretics, but most act by decreasing the amount of fluid that is reabsorbed by the tubules of the kidneys, whence the fluid passes back into the blood. The most widely used diuretics, the benzothiadiazine (*e.g.,* chlorothiazide), interfere with the reabsorption of salt and water by the kidney tubules. Instead of being reabsorbed, the salt and water are ultimately excreted, thus increasing the flow of urine. After they were synthesized in the late 1950s, the benzothiadiazine replaced most other existing diuretics. They are more convenient than some other diuretics in that they can be taken orally in the form of pills. These drugs are also used to reduce high blood pressure (hypertension).

SEDATIVE ACTIVITY

A drug that calms a patient, easing agitation and permitting sleep. Sedatives generally work by modulating signals within the central nervous system. If sedatives are misused or accidentally combined, as in the case of combining prescription sedatives with alcohol, they can dangerously depress important signals that are needed to maintain heart and lung function. Most sedatives also have addictive potential. For these reasons, sedatives should be used under supervision and only as necessary.

They are central nervous depressants and interact with brain activity causing its deceleration. Various kinds of sedatives can be distinguished, but the majority of them affect the neurotransmitter gamma-amino butyric acid (GABA), which are brain chemicals performing communication between brain cells. In spite of the fact that each sedative acts in its own way, they produce beneficial relaxing effect by increasing GABA activity.

At higher doses it may result in slurred speech, staggering gait, poor judgment, and slow, uncertain reflexes. Doses of sedatives such as benzodiazepines, when used as a hypnotic to induce sleep, tend to be higher than amounts used to

relieve anxiety, whereas only low doses are needed to provide a peaceful effect. Sedatives can be misused to produce an overly-calming effect (alcohol being the classic and most common sedating drug). In the event of an overdose or if combined with another sedative, many of these drugs can cause unconsciousness (see hypnotic) and even death[10].

TERMINOLOGY

There is some overlap between the terms "sedative" and "hypnotic". Advances in pharmacology have permitted more specific targeting of receptors, and greater selectivity of agents, which necessitates greater precision when describing these agents and their effects: Anxiolytic refers specifically to the effect upon anxiety. (However, some benzodiazepines can be all three: sedatives, hypnotics, and anxiolytics). Tranquilizer can refer to anxiolytics or antipsychotics. Soporific and sleeping pill are near-synonyms for hypnotics.

The term "chemical cosh"

The term "chemical cosh" (a club) is sometimes used popularly for a strong sedative, particularly for: Widespread dispensation of antipsychotic drugs in residential care to make people with dementia easier to manage. Use of Ritalin to calm children with attention deficit hyperactivity disorder, though paradoxically this drug is known to be a stimulant[11].

AIM AND OBJECTIVES

AIM

The Aim of the present study is to evaluate the Diuretic and Sedative activity of ethanolic leaves extract of *Basella alba.*

OBJECTIVES

The Objective of the study was:

- To prepare the ethanolic leaves extract of *Basella alba* Leaves.
- To study the diuretic activity and sedative activity of Ethanolic leaves extract of *Basella alba* using following models.
    a) Diuretic activity by LIPSCHITZ TEST
    b) Sedative activity by MOTOR COORDINATION TEST

PLAN OF WORK

I. Literature review
II. Phytochemical evaluation.
    a. Collection of plantmaterial
    b. Identification and Authentication
    c. Extraction
    b. Qualitative phytochemical analysis
III. In-vivo studies.
    i. Acute toxicity study
    ii. Efficacy studies
        a) Diuretic activity of LIPSCHITZ TEST.
        b) Sedative activity by MOTOR COORDINATION TEST.

MATERIALS AND METHODS

DESCRIPTION

Basella alba is a fast-growing, soft-stemmed vine, reaching 10 metres (33 ft) in length. Its thick, semi-succulent, heart-shaped leaves have a mild flavour and mucilaginous texture. It is a fast growing perennial climber, growing up to 9 m in length and belongs to the family *Basellaceae*. It is a valuable vegetable that can be cultivated from either seed or cutting. The stem is green or purplish and quadrangular in shape, about 2 to 3 cm thick, with prominent nodes and internodes. The taste is bland and mucilaginous, with no odour. The leaves are fleshy and ovate or heart shaped, chordate base, dark green in colour, glossy above and glaucous below. The size of the leaves varies from 3 to 9 cm in length and 4 to 8 cm in width. Taste was found to be bland, with no odour. The flowers are inconspicuous, bisexual white flowers borne on axillary spikes or branching peduncles[42],its fruits are fleshy and stalk less, ovoid or spherical in shape, 5 to 6 mm, 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. It is rich in vitamins A and C, iron and calcium. It has been shown to contain certain phenolic phytochemicals and it has antioxidant properties[43].

It is also called Malabar Spinach. There are two varieties - green and red. 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 500 metres (1,600 ft) above sea level. The plant is native to tropical Asia[5]. It grows best in sandy loam soils rich in organic matter with pH ranging from 5.5 to 8.0[44,45].

PLANT PROFILE

Figure no: 1 and 2

Kingdom: Plantae Order: Caryophyllales
Botanical name: Basella alba linn.var.rubra stewart.
Species: Basella alba Linn
Family: Basellaceae
Sub Family: Baselleae
S ynonym: Basella cordifolia Linn.
Genus: Basella

MEDICINAL USE

Leaves of *Basells alba* is used for the treatment of hypertension and externally in treatment sores, urtricaria and gonorrhoea. antifungal, anticonvulsant, analgesic, anti-inflammatory and androgenic activities, demulcent, febrifuge, and laxative properties. It is beneficial to drink it during fluid retention, dysentery[46]. diarrhoea, constipation and catarrh. Anti cancer, Anti viral, Anti bacterial, Anti microbial. Antioxidant, Anti depreesent, skeletal muscle relaxant. Diuretic[47], anti-ulcer activity. Anemia in women, anticancer such as melanoma, leukemia and oral cance[48].

PHYTOCHEMICL 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 50 gm 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 until further studies[48,49,50,and52].

Figure 3: Basella Alba Extraction by Using Soxhlet Apparatus

TESTS FOR ALKALOIDS

- **Mayer's Test:** 2ml solution of the extract and 0.2 ml of dilute di-hydrochloric acid were taken in a test tube. Then 1ml of Mayer's reagent was dissolved. Yellow color precipitate was formed and that was indicated as the presence of alkaloids[53].
- Dragendroff's Test: 2ml solution of the extract and 0.2ml of dilute hydrochloric acid were taken in a test tube. Then 1ml of Dragendroff; s reagent was added. Orange brown precipitate was formed and that was indicated as the presence of alkaloids[54].Test for Carbohydrates[66].
- **Molisch's Test:** Test solution with 2 ml of molisch's reagent (α-naphthol solution in alcohol) and 2 ml of concentrated sulphuric acid was added slowly from the sides of the test tube. It was observed for violet ring at the junction of two liquids.
- **Fehling's Test:** 1 ml of fehling's A and B solutions was mixed and boiled for one min. Equal volume of test solution was added. Heated in boiling water bath for 5-10 min and observed for first yellow, then brick red precipitate.
- **Benedict's Test:** Equal volume of benedict's reagent and test solution were mixed in the test tube. Heated in boiling water bath for 5 min. Solution may appear green, yellow or red depending on amount of reducing sugar present in test solution.

TESTS FOR TANNINS

Ferric Chloride Test

5ml solution of the extract was taken in a test tube. Then 1ml of 5% ferric chloride solution was added. Greenish black precipitate was formed and indicated the presence of tannins[55].

Test for Flavonoids

Added a few drops of concentrated hydrochloride acid to a small amount of an alcoholic extract of the plant material. Immediate development of a red color indicates the presence of Flavonoids[56].

Tests for Saponins

1ml solution of the extract was dilute with water to 20ml and shaken in a graduated cylinder for 15minutes. No one-centimeter layer of foamindicates the absence of saponins. Following reagents are used for different chemical group test[57].

Test for Mucilage: 0.5gm of mucilage was hydrolyzed with 50ml of 0.1N sulphuric acid. The solution was neutralized using barium carbonate and filtered. The filterate was concentrated and subjected to thin layer chromatography on silica gel G plate (Merck). Mobile phase were used n-butanol, ethanol and water (10:2:2) resp. The spots were visualized with aniline phthalate reagent as dark brown spot[58].

Test for phenols

- **Ferric chloride test:** The fraction of extract was treated with 5 % ferric chloride and observed for formation of deep blue or black colour[59].

Tests for Glycosides

Liebermann's test

- 2 ml of acetic acid and 2 ml of chloroform mixed with entire plant crude extract. The mixture was then cooled and added H2SO4 concentrated, green color indicated the entity of aglycone steroidal part of glycosides[60].

Salkowski's test

- H2SO4 concentrated (about 2 ml) was added to the entire plant crude extract. A reddish brown color produced indicated the entity of steroidal aglycone part of the glycoside[61].

Keller-kilani test

- A mixture of Acetic acid glacial (2 ml) with 2 drops of 2% FeCl3 solution was added to the plant extract and H2SO4 concentrated. A brown ring produced between the layers which indicated the entity of cardiac steroidal glycosides[62].

Test for Anthocyanin

NaOH test

- A small amount of extract was treated with 2M NaOH and observed for the formation of blue green colour[63].

IN VIVO STUDIES

Animals used

Adult wistar albino male rats (150-180g) were procured from the laboratory animal model house, Hindu College of Pharmacy, Guntur, Andhra Pradesh, India and used in the study. The animals were kept under standard environmental conditions of room temperature ($22^{o}\pm2^{O}C$), relative humidity (50%±5%) and 12h light and dark cycle. The animals were housed in the colony cages (three rats per cage) and provided feed (commercial pellets contain a balanced ration obtained from the Vyas labs, Hyderabad) 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. The study was conducted after obtaining ethical committee clearance from the Institutional Animal Ethics Committee No: HCOP/IAEC/PR-1/2018.

METHOD

Acute toxicity studies

Three wistar rats were selected for the study. The overnight asted 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[63].

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. They were deprived of food and water for 16 hr prior to the 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[63, 64].

The treatment was as follows

Group I- control. (Normal saline 25ml/kg; p.o)
Group II- furosemide.(20mg/kg; p.o)
Group IIII- *Basella alba* (Test drug 100mg/kg;p.o)
Group IV- *Basella alba* (Test drug 200mg/kg;p.o)

STASTICAL ANALYSIS

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

Assessment of Sedative Activity

Test for Motor coordination :(Rota rod test)

The Rota rod test is used to look at the effect of a drug on the motor coordination of the animal. As sedation causes loss of motor coordination this test can be used to evaluate the sedative activity of the drug. The mices were divided into 4 groups of 6 animals each. The apparatus was turned on at the speed of 20 rotations per minute. Mice was placed one by one on the rod and the fall off time is recorded i.e. when the mouse falls off from the rod. A normal mouse usually falls off within 3-5 minutes. The fall off time was recorded. The first group received normal saline;

p.o (control) the second group received the standard drug diazepam at 1mg/kg, i.p. The test drug was studied at two concentrations (100 and 200mg/kg, p.o) to the third and fourth groups respectively. Thirty minutes after the vehicle or test drug administration, each mouse was placed on the rod for 180 s where diazepam was given 15 min before the experiment. Then the fall off time was again recorded one hour after oral drug administration and thirty minutes after i.p. The percentage decreases in fall off time were compared[65].

The treatment was as follows

Group I- control. (Normal saline 25ml/kg; p.o)
Group II- furosemide.(20mg/kg; p.o)
Group IIII- *Basella alba* (Test drug 100mg/kg;p.o)
Group IV- *Basella alba* (Test drug 200mg/kg;p.o)

RESULT 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^{O} 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.

Table 1- Percentage yield of Ethanolic extract of leaves of Basella alba extract.

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

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates, saponins, tannins, flavonoids, phenols, steroidal glycosides, mucilage, anthocyanin in *Basella alba* extract.

Table2- Preliminary phytochemical analysis of ethanolic 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	–

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.

1. DIURETIC ACTIVITY

It was observed that BAE (*Basella alba* ethanolic leaves extract) has shown significant ($p<0.05$) diuretic activity by increasing urine out put and increased out put 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 200 mg/kg ; p.o) studied, higher dose (200 mg/kg ; p.o) produced more effect. A comparison was made with the standard furosemide. (table- 3&4, Graph -1& 2)

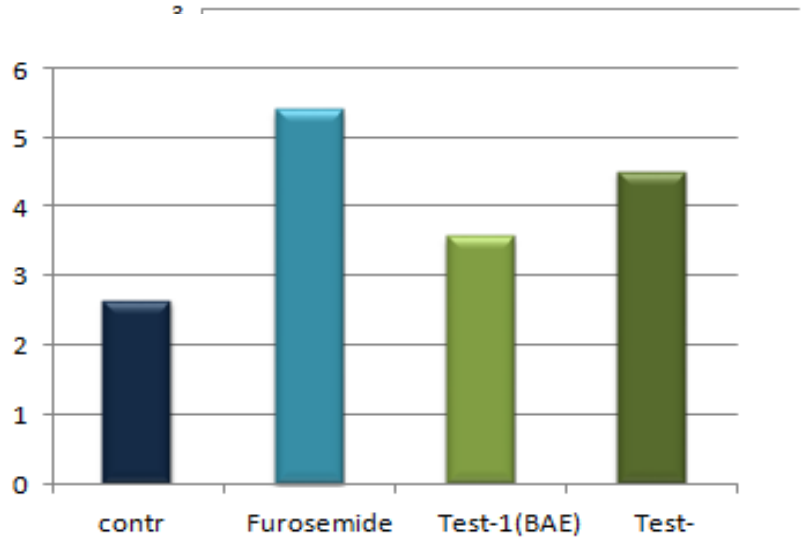
Figure4: Fabricated metabolic cage

Diuretic activity of BAE (*Basella alba extract*) on urine volume Table-3: Diuretic activity of BAE *(Basella alba extract)* 0n urine volume

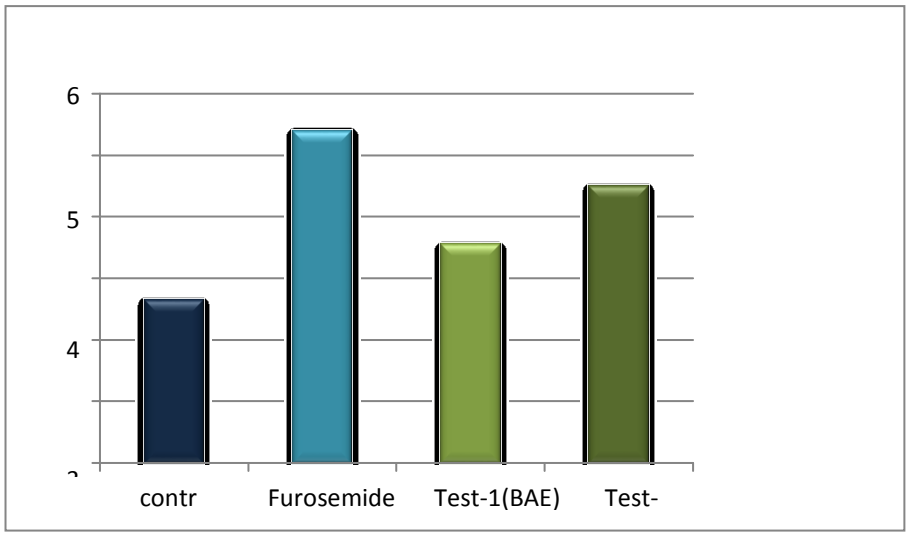
Group	Treatment	Dose Mg/kg	Urine vol(m) 5hrs	Diuric index	Urine vol(ml) 24hrs	Diuretic index
1.	Control	-	0.60	0.99	2.73	0.97
2.	Furosemide	20	2.95 ± 0.93*	1.04	5.01 ±0.57*	1.0
3.	BAE	100	1.20 ± 1.25*	1.36	3.76 ± 1.4*	1.13
4.	BAE	200	2.06 ± 0.65*	1.65	4.70 ± 0.36*	1.45

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.1. Effect of BAE on urine volume at 5hrs.



Graph.2. Effect of BAE on urine volume at 24hrs.



Diuretic activity of BAE on electrolytes

Table 4. Diuretic activity of 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	BAE	100	138.7 ±2.4*	5.26±1.2*	102.7 ±0.6*	1.02	1.46	1.20
4	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.

SEDATIVE ACTIVITY

Our results demonstrated that treatment with BAE (*Basella alba*) at 100, 200 mg/kg doses significantly ($p<0.05$) reduced the falling latency of the animals from the rotating rod. The decrement of the latency was calculated as 41.98, and 65.05% of the control, 100, 200 mg/kg doses of BAE respectively (Table-5). A comparison was made with control and standard (Diazepam).

Sedative activity of BAE (Basella alba extract) on Motor coordination of mice

Table 5. Effect of BAE on Motor coordination of mice

S.No	Treatment	Dose mg/kg	Responses	
			Rota rod performance (fall of time in sec)	%inhibition
1.	control	0.1ml/mice	110.5	0
2.	Diazepam	1	25.6 ± 3.2 *	75.76
3.	BAE	100	65.6 ± 2.5 *	41.98
4.	BAE	200	40.6 ± 3.7 *	65.05

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.3. Effect of BAE on Rota-rod performances (fall of time) of mice.

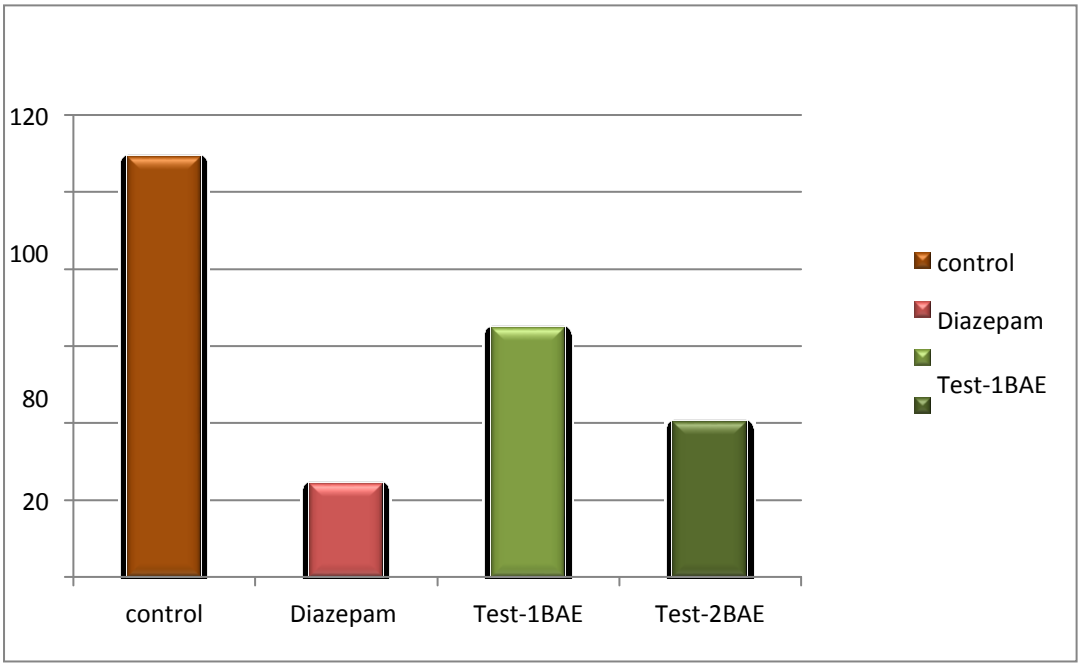


Figure 5: Effect of control on Rot-arod performances

Figure 6: Effect of Diazepam on Rota-rod performances of mices

Figure 7: Effect of BAE on Rota-rod performances of mices

Figure 8: Effect of BAE on Rota-rod performances of mices

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 LD_{50} of the BAE will be > 2000mg/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 stastically significant dose dependent increase in the volume of urine and excretion of electrolytes hence BAE has Diuretic activity.The Rota rod test is used to look at the effect of a drug on the motor coordination of the animal. As sedation causes loss of motor coordination this test can be used to evaluate the sedative activity of the drug. The studies on sedative activity model reveled that all the BAE treated groups decreased fall of time compared to control. Hence the BAE possessed sedative activity also.

SUMMARY

The present study was aimed to assess the Diuretic and Sedative activity of Etanolic *Basella alba* Leaf extract. The plant material was collected, Authentified 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 Etanolic leaf extract of *Basella alba* was assessed by the method previously described by Lipschitz etal. The urine out and potassium, sodium, chloride levels in the urine were measured. The Sedative activity of ethanolic leaf extract of *Basella alba* was assessed by the Motor coordination activity model (or) Rot rod test. In which we measured the fall time of mices on Rota rod was measured. Then finally percentage decrease in fall off time was compared with,control,and,standard,(Diazepam).

CONCLUSION

The present findings in our study indicate that ethanolic extract of leaves of Basella alba (BAE) possesses diuretic and sedative activity. However, further studies will be needed to isolate bioactive compound(s) and elucidate the precise molecular mechanisms responsible for the pharmacological activities of the plant.

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