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PHARMACOLOGICAL SCREENING OF DIKAMALIARTANE-A, A CYCLOARTANE ISOLATED FROM GUM RESIN, DIKAMALI

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ABSTRACT: The biological activitites of Dikamaliartane-A, a cycloartane isolated from gum resin Dikamali of *Gardenia gummifera*/*Gardenia lucida* was screened for some pharmacological actions. The study was carried out using albino mice (20-25gr). It reduced locomotor activity and potentiated pentobarbitone-induced sleeping time in mice indicating Central Nervous System depressant activity. It protected mice from strychnine and electro shock–induced convulsions indicating that it has anti-convulsant activity. All these activities were statistically significant. The LD_{50} (Lethal Dose) was carried out in mice according to Organization for Environmental Cooperation and Development (OECD) Guidelines 423. The LD_{50} was tested in three mices for each dose with doses from 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg. The LD_{50} was found to be 500mg/kg.

Keywords: Dikamaliartane-A, Cycloartane, Dikamali, Locomotor activity, Central Nervous System depressant activity, Anticonvulsant activity and OECD Guidelines 423.

INTRODUCTION

Gardenia gummifera Linn. and *G.lucida* Roxb., both belonging to the family Rubiaceae, are medium sized trees growing all over India. The gum-resin oozing out from the leaf buds of these trees is called Dikamali. It is marketed in the form of lumps/ cakes which are greenish yellow in color. It has an offensive odour and sharp pungent taste. Dikamali is claimed to have a number of medicinal properties which include antispasmodic, carminative, anthelmintic, diaphoretic and expectorant. It is also claimed to be useful in dyspepsia, flatulence for cleaning foul ulcers and wounds, and to keep off flies from wounds in veterinary practice (R.N.Chopra et al., 1956, K.R. Kirtikar and B.D.Basu , 1987, P.S.Varier , 1995). The leaf extract of *G.gummifera* is a component of an ayurvedic medicine by name "unmadanashak ghrita" which is indicated for the treatment of mania, epilepsy and other CNS disorders (G.S .Achliya et al., 2004). A number of Flavanoids were isolated from Dikamali in the past (A.V. Rama Rao et al., 1970, M. Krishnamurthi et al., 1972, S.R. Gupta et al., 1975, S.C. Chhabra et al., 1976, S.C.Chhabra , et al., 1977, S.C. Chhabra et al., 1977, M.Krishnamurthi et al., 1971, K.K. Purushothaman et al., 1973). Recently, a number of new cycloartanes were reported from this source of which Dikamaliartane A is the main cycloartane (O. Kunert et al., 2009).

Diethylether extract of Dikamali (*G. gummifera*) was found to have analgesic, anti-inflammatory, antipyretic and anthelmintic activities. Also, it exhibited good anti-oxidant activity (S.K.Sridhar et al., 2003). Ethanolic extract of *G. gummifera* gum resin was found to possess cholesterol suppressive capacity and antioxidant activity. It was also found to attenuate the accelerated development of atherosclerosis in hypercholesterolemic rats (A.V.Gajjar et al., 2008). Ethanolic extract of *Gardenia gummifera* gum resin was found to have *in vitro* anthelmintic activity (M.A.Kamble et al., 2008).

Cycloartanes and flavanoids isolated from the aerial parts of some of the related *Gardenia* species were found to possess Cytotoxic and anti-HIV activities (P.Tuchinda et al., 2004, V. Reutrakul et al., 2004, P.Tuchinda et al., 2002, G.L. Silva et al., 1997, T.Nuanyai et al., 2009, T.Nuanyai et al., 2010). This study was undertaken to screen the main cycloartane of Dikamali, Dikamaliartane-A for some of its pharmacological actions. This is the first report of the screening of Dikamaliartane-A for its actions.

Experimental

Dikamaliartane-A was isolated in our UCPSc Pharmaceutical Chemistry lab Kakatiya University and its structure was established (Fig. 1) (O. Kunert et al., 2009).

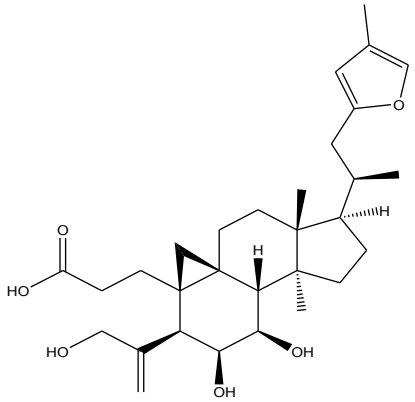

Fig. 1: Structure of Dikamaliartane-A

CAS name: (6β, 7β, 17R, 20R) – 6,7,29 Trihydroxy- 23, 26 epoxy-3,4- secocycloartan-4(28),23(24),25(26) trien-3-oic acid.

Chemicals: Pentobarbitone sodium and Strychnine Hydrochloride were procured from Sigma Aldrich, MI, USA. Phenobarbitone sodium injection 200mg/ml was purchased from Piramal H.C., Mumbai and Diazepam 10mg/2ml (Calmpose) injection was purchased from Ranbaxy (Pharma), New Delhi.

Experimental Animals

Albino mice (mean weights in the range of 20–25gms) were obtained from Mahaveer Enterprises, Hyderabad, India and housed in polypropylene cages in a room where the congenial temperature was 27±1°C and 12 hrs light and dark cycles were maintained. The animals were allowed to acclimatize to the environment for 7 days and supplied with a standard pellet diet and water ad libitum. All procedures using animals were reviewed and approved by the Institutional Animal Ethical Committee of Kakatiya University, Warangal, India.

Acute toxicity studies

This was performed as per OECD guidelines 423. Four groups of 3 mice in each group at the doses of 5mg/kg, 50mg/kg, 300mg/kg, 2g/kg were administered and mortality was observed.

Gross behavioral studies

Locomotor activity of Dikamaliartane-A in mice was measured using an actophotometer mice were divided into two groups of 6 animals each. Group I was treated with 1% gum acacia and group II was treated with Dikamaliartane-A 30mg/kg. The basal activity (score) was recorded for 5 min at 0, 1 and 2 hrs after administration of test compound using actophotometer (S.K. Kulkarni , 1999).

Effect of Dikamaliartane-A on Pentobarbitone-Induced Sleeping Time in mice

The mice were divided into three groups of six animals each. Group I serves as control; group II and III receive Dikamaliartane-A at a dose level of 10 and 30 mg/kg suspended in 1% gum acacia solution (i.p) respectively by intra peritoneal route. After 30 minutes of administration of test samples, pentobarbitone (35 mg/kg, i.p) was administered to all the groups. The time between the loss of righting reflex and the regain of this reflex was measured as the sleeping time and also onset of sleep, duration of sleep were noted (S.K.Kulkarni , 1999).

Evaluation of Anti epileptic activity

Strychnine (STR)-induced seizure

Male Swiss albino mice weighing 20-25g were randomly divided into 3 groups (n=6). Group I served as control (received 1% gum acacia); Group II received Diazepam (5mg/kg, i.p) (N.S.Parmar and Shiv Prakash, 2006) and served a positive control, where as group III received Dikamaliartane-A (30 mg/kg in 1% gum acacia, i.p). Strychnine (STR; 2 mg/kg; i.p) was administered to all the animals after 30 min of test or standard drug administration. The animals were observed for onset of myoclonic spasm and clonic convulsion up to 30 min after STR injection. The percentage of protection was observed and recorded.

Electroshock induced seizures

In the electroshock-induced seizure experiment, the maximal electroshock (MES) method was used. In brief, tonic convulsions of the hind extremities of the mice were observed. The animals were divided randomly into 3 groups containing 6 animals each. Group I served as vehicle control group received 1% gum acacia solution; group II served as positive control and treated with phenobarbitone (20mg/kg; i.p) and group III served as test and received 30mg/kg in 1% gum acacia; i.p. Thirty minutes after administration of test or standard drugs, electroshock (induced by passing alternating current of 50 Hz and 50 mA for 0.2 sec through corneal electrodes) was applied to induce convulsion. Phases of electroshock-induced convulsions like tonic flexion, tonic extensor, clonic convulsions, stupor and. recovery (or) death were determined for each group (S.K.Kulkarni, 1999).

Statistical analysis

All the data was expressed as Mean ± SD. One-way analysis of variance (ANOVA) and Dunnett test were used to compare means from the control group and each of the groups exposed to test or standard solutions and the statistical significance was judged at the 0.05 probability level.

RESULTS

Acute toxicity studies

The acute toxicity study of Dikamaliartane-A was determined in mice. When a dose of 2g/kg of Dikamaliartane-A was administered all three mice were died. At a dose of 300mg/kg no death of mice were recorded. From the data the LD_{50} of Dikamaliartane-A was determined to be 500mg/kg following OECD guidelines 423.

Gross behavioral studies

Locomotor activity of Dikamaliartane-A in mice was measured using an actophotometer. There was a significant reduction in the locomotor activity in mice at 1 hr in the treated group of mice ($p<0.0001$ at a dose of 30 mg/kg of Dikamaliartane-A) when compared with control group. The results are shown in table 1.

Table 1: Gross behavioral studies

S. No.	Before Treatment	After Treatment	
	0 hr	1hr	2hr
1	315	140	160
2	430	149	158
3	409	199	233
4	360	135	180
5	402	144	250
6	418	210	293
Mean	389	143	199
SD	43.38	56.99	75.88
		p< 0.001	p< 0.01

Effect of Dikamaliartane-A on Pentobarbitone- Induced Sleeping Time in mice

Dikamaliartane-A produced a dose dependent increase in the pentobarbitone induced sleeping time in mice. It was found to be significant with $P<0.05$ at 10mg/kg and $p<0.001$ at 30mg/kg. There was also reduction in the onset of sleeping time when compared with control group. The results are shown in table 2.

Table 2: Effect of Dikamaliartane-A on Pentobarbitone induced sleeping time

Group/Treatment	Onset of sleep (min)	Duration of sleep (min)
Group-I (Control)	7.2 ± 0.84	36.8 ± 9.78
Group-II (Dikamaliartane-A; 10mg/kg)	5.1 ± 1.22**	54.8 ± 18.07*
Group-III (Dikamaliartane-A; 30mg/kg)	4.8 ± 0.84***	62.2 ± 7.26***

Data was expressed as mean ± SD (n=6);
*p<0.05;
**p<0.01;
***p<0.001 vs control

Anti-convulsant activity

A. Effect of Dikamaliartane-A on strychnine induced convulsions in mice

Dikamaliartane-A increased time of onset of convulsions from 5.2±0.44 min in the control group to 17.0 ± 1.87 min at a dose of 30 mg/kg body weight ($p<0.05$). The effect was less, when compared with that of the standard drug used for comparison i.e., diazepam at a dose of 5mg/Kg body weight ($p<0.001$). Similarly, Dikamaliartane-A offered 83.3% protection against mortality in this experiment, while diazepam offered 100% protection in the dose tested. Results are shown in Table 3.

Table 3: Effect of Dikamaliartane-A on strychnine induced convulsions in mice

Group/Treatment	Onset of convulsions (min)	% mortality	% protection
Group-I (Control)	5.2 ± 0.44	66.6	33.3
Group-II (Diazepam; 5mg/kg)	17.0 ± 1.87**	0	100
Group-III (Dikamaliartane-A; 30mg/kg)	9.0 ± 3.81*	16.7	83.3

Data was expressed as mean ± SD (n=6);
*$p<0.05$.
**$p<0.001$ vs control.

B. Effect of Dikamaliartane-A on electroshock- induced convulsions in mice

Dikamaliartane-A protected mice against electro shock- induced convulsions at a dose of 30 mg/kg body weight. There was 100% protection against mortality. Results are presented in Table 4.

Table 4: Effect of Dikamaliartane-A on electroshock- induced convulsions in mice

Group/Treatment	Tonic flexion	Clonic extensor	Stupor	% mortality	% protection
Group-I (Control)	3.2±1.09	15.6 ± 1.34	138.8±18.7	66.67	33.33
Group-II (Phenobarbitone; 20mg/kg)	-	-	14± 10.83***	0	100
Group-III (Dikamaliartane-A; 30mg/kg)	1.6 ± 0.89*	15 ±10.0	45 ± 23.18**	0	100

Data was expressed as mean ± SD (n=6);
*$p<0.05$.
**$p<0.01$ vs control
***$p<0.001$ vs control

DISCUSSION

The LD_{50} of Dikamaliartane-A was determined to be 500mg/kg body weight in mice. It reduced the locomotor activity at a dose of 30 mg/kg significantly ($p<0.001$). It increased pentobarbitone-induced sleeping time. There was also a reduction in the onset of sleeping time in the doses tested. These effects were significant, dose dependant and indicate that Dikamaliartane-A has CNS depressant activity.

Dikamaliartane-A exhibited anticonvulsant activity when tested by strychnine- induced and electro- shock induced convulsions in mice. The activity was significant in the dose tested (30mg/Kg). In the strychnine–induced convulsions model the onset of convulsions were delayed and the percentage protection against the mortality was 83.3%. In the electroshock–induced model, Dikamaliartane-A afforded not only 100% protection against the mortality, but also reduced the period of tonic flexion and stupor. The results of these experiments clearly indicate that Dikamaliartane-A has anticonvulsant activity.

Dikamaliartane-A is a rare cycloartane with ring–A being seco and ring-D containing a methylfurane moiety in the side chain. This is the first report of the CNS effects of Dikamaliartane-A.

Conclusion

Dikamaliartane-A exhibited CNS depressant and anticonvulsant properties.

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