

EVALUATION OF SYNERGISTIC EFFECT OF METHANOLIC LEAVES EXTRACT OF *HYGROPHILA DIFFORMIS* ON PROLONGATION OF SLEEPING TIME ALONG WITH THIOPENTONE SOD. - A COMPARATIVE STUDY WITH DIAZEPAM.

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Abstract

The objective of the study was to evaluate the synergistic effect of methanolic extract of leaves of *H. difformis* on prolongation of sleeping time along with thiopentone sod. as compare with diazepam. The material was dried in shade, they were powdered and extracted with methanol. Preliminary phytochemical test were done. The methanolic extract showed presence of phenolic compounds, flavonoids, tannins, steroids & also alkaloids. . The methanol extract at an IP dose of 200 mg/kg and 400mg/kg exhibited a significant effect ($p < 0.01$) on thiopentone sod. induce sleeping time, where as 400mg/kg dose of *H. difformis* showed greater effect which is comparable with diazepam (4mg/kg). Our findings suggested that methanolic leave extract of *H. difformis* possessed hypnotic effect in the dose dependent manner.

Keywords: *Hygrophila difformis*, Thiopentone sod., Sleeping time, Diazepam, Hypnotics

Introduction:

Ethno pharmacological approach to drug discovery is great value in developing countries, since scientific validation of a local remedy may encourage its use and introduction into therapy in its original habitat[1]. Efficacy and safety are evaluated and potential harm is minimized through a selected method of preparation of the plant materials. India has a rich sources of biodiversity (both flora and fauna), possessing tremendous medicinal properties[2]. Scientist are searching newer avenues and material for drug development and for that they are search for new medicinal plants, which are till date not have been exploited to their fullest extent (except traditional systems). The rich biodiversity of the tropical region all over the world have attracted scientific attention and tropical region of lower plane land of West Bengal is not an exception of that trend. *Hygrophila difformis* (family- acanthaceae) is a tropical

plant, widely distributed along plane land of uluberia and being a medicinally important plant as reported from several local villagers in that area. Accordingly in our laboratory we have chosen this plant for the study of hypnotics effect to fulfill our quench of thirst and to reveal whether this plant could be utilized as an effective source for future medicine development.

Materials and methods:

Plant Materials

The plant *Hygrophila difformis* (Family: Acanthaceae) was collected from Uluberia Sub-Division of Howrah district, West Bengal, India in the month of November. The plant material was taxonomically identified by the taxonomists of Botanical Survey of India, Kolkata. A voucher specimen (NO.CNH/I-I (291)/2009/Tech.II/333) has been preserved in our laboratory for future reference.

Experimental Animals

Swiss albino mice (20-22 g) housed at normal laboratory conditions ($24 \pm 2^{\circ}\text{C}$) for at least ten days where used for

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pharmacological experiments. The animals were supplied standard pellet diet and water ad libitum.

Extraction procedure

The leaves were dried under shade and then powered with a mechanical grinder to obtain a coarse powder, which was then subjected to soak in petroleum ether overnight and subsequently packed in a Soxhlet apparatus using methanol (60⁰-65⁰C), and methanol extract (17 % w/w yield) . The resulting methanol extract was then used for preliminary phytochemical testing.

Acute toxicity test [Determination of lethal dose (LD50)]

LD₅₀ value was estimated by "Acute Toxicity Test". The LD₅₀ was determined by the method of Litchfield and Wilcoxon[3]. Swiss albino mice, Weighting between 25 g were used for the study. Dose of (1000mg/kg) the methanol extract of *Hygrophila difformis* leaf was dissolved in normal saline and injected intraperitoneally to three different groups of mice, each group containing 6 animals, which were fasted for 18 hours, the animals were observed 24 hours.

Thiopentone sodium induces sleeping time:

The method employed in this study was as described by Vogel[4].The mice were divided into four groups of six animals each. Thiopentone sod. Was introduced intraperitoneally to all the groups at the dose of 40mg/kg body weight. The test compound (*Hygrophila difformis*) was injected intraperitoneally in a dose of 200 and 400 mg/kg and one group received compared drug diazepam in a dose of 4mg/kg 30 minutes before Thiopentone sod. Injection. The control group was received normal saline. The onset time of sleeping was noted of all animals. After induction of sleep mice was placed inverted position and when sedation was over mice comes to normal posture time was noted. The duration of sleep was calculated of all animals.

Statistical Analysis:

Results were expressed as mean \pm S.E. The statistical evaluation was done by analysis of variance (ANOVA) coupled with student's t-test, p<0.05 was considered to be statistically significant.

Result & Discussion:

The results of the preliminary phytochemical are as follows:

Test for methanolic extract	Result		
	(+)	(-)	
Test for alkaloid			
1. Mayer's test	(+) (+) (+)		
2. Dragendroff's test	(+) (+) (+)		
3. Hagger's test	(+) (+)		
Test for tannin (Extract with 5% FeCl ₃ solution.)	(+) (+) (+)		
Test for flavonoid			
1. Extract with alcohol + neutral FeCl ₃ solution.	(+) (+) (+)		
2. Extract with alcohol + lead acetate.	(+) (+) (+)		

Acute toxicity test (Determination of lethal dose, LD50):

The methanolic extract of *Hygrophila difformis* leaves upto a dose of 1000mg/kg (i.p.) was found to be tolerated and no lethality was observed. Dose range of (200-400) mg/kg (i.p.) was selected for the present investigation.

Effects of extract on thiopentone induce sleeping time:

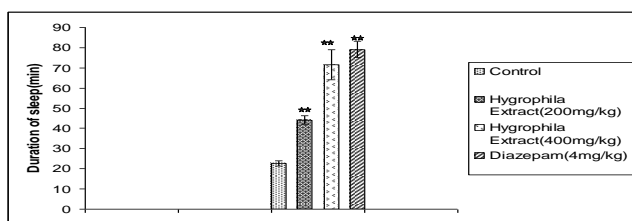
The extract (200mg/kg; i.p. & 400mg/kg; i.p.) prolong the thiopentone induce sleeping time. Moreover the effect of

extract was found to be comparable to that of diazepam. (Table1; Fig1)

Table 1: Effect of *Hygrophila difformis* leaf extract on thiopentone induces sleeping time:

Animal No.	Duration of sleep(min)			
	Control + Thiopentone Sod.(40mg/kg)	Hygrophila Extract(200mg/kg) + Thiopentone Sod.(40mg/kg)	Hygrophila Extract(400mg/kg) + Thiopentone Sod.(40mg/kg)	Diazepam (4mg/kg) + Thiopentone Sod.(40mg/kg)
1.	25	45	71	73
2.	23	51	104	95
3.	20	49	58	82
4.	27	40	63	69
5.	18	42	78	71
6.	23	38	55	84
Mean ± SEM	22.66±1.33	44.16±2.09	71.5±7.38	79±4.05
P	—	<0.01	<0.01	<0.01

Fig 1: Effect of *Hygrophila difformis* leaf extract on thiopentone induce sleeping time (mean ± SEM; n=6).P<0.01**



The methanolic leaf extract of *H.difformis* extract was assessed for hypnotics activity, and found to prolong the thiopental sodium– induced sleeping time in mice. A depressant effect of an extract on the CNS is indicated by a prolongation of the thiopentone-induced sleeping time[5,6].Extract of dose (200mg/kg) significantly ($p<0.01$) prolong the time of sleep and also extract of dose (400mg/kg) exhibited greater activity which was comparable to standard drug diazepam (Fig1). Flavonoids could act as a benzodiazepine like molecules in the CNS and modulate the GABA- generated chloride current in the animal models of sedation& convulsion. It is possible that hypnotics activity of the extract related to its constituent, flavonoid[7].

Conclusion:

The present investigation indicates that methanolic extract of hygrophila leaf exert significant synergistic effect with thiopentone sod. and dose of 400mg/kg have remarkable hypnotics effect which is comparable with diazepam.

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