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Research Article

POTENTIATION OF THIOPENTONE SODIUM INDUCED HYPNOSIS BY BERBERIS ARISTATA IN RODENTS

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ABSTRACT

Berberine is an isoquinoline alkaloid present in roots, stem, bark and fruits of *Berberis* species. Berberine based formulations are widely used in traditional systems of medicine including Ayurveda and Traditional Chinese Medicine for at least 3000 years. Berberine has demonstrated a wide range of pharmacological activities incld. antimicrobial, hepatoprotective, ionotropic, anti-arrhythmic, hypolipidemic and anti inflammatory. Central nervous system activities of Berberine have been demonstrated in different animal models. The present study investigated the effects of root extract of *Berberis aristata* plant using righting reflex in mice. Three doses of the extract (5, 10, 20 mg/kg) were injected i/p in comparison with diazepam (3mg/kg) as positive control and saline as negative control. After 30 min of injection of extract, thiopentone sodium (40mg/kg) was injected and increase in sleeping time by extract was recorded. The results showed that the lower doses (5 mg/kg) of the extract did not affect the locomotor activity and thiopentone induced sleeping time where as the dose of 20 mg/kg, significantly reduced the motility and locomotor activity. There was significant motor incoordination and hypnosis was prolonged to 167.83±52.127 min (p=0.004) as compared to diazepam 28.67±9.812 min (p=0.054).

Keywords: Hypnosis, Thiopentone sodium, mice, *Berberis aristata.*

INTRODUCTION

Indian Barberry (*Berberis aristata*, *Berberis* species) is an erect; 2-3 meters high spinous shrub found growing wild in the Sub Himalayan tract, Nilgiris and Ceylon. The species is popularly known as "Kashmal", "Rasont", "Chitra", "Simlu" and "Darhaldi" [1]. It is one of the very important medicinal plants. Almost every part of the plant has some medicinal value. Berberine is an isoquinoline alkaloid present in roots, stem, bark and fruits of *Berberis* species. Berberine based formulations are widely used in traditional systems of medicine including Ayurveda and Traditional Chinese Medicine for at least 3000 years. Berberine has demonstrated a wide range of pharmacological activities incld. antimicrobial[2,3], hepatoprotective[4], ionotropic[5,6], anti-arrhythmic[7], hypolipidemic[8,9,10] and anti inflammatory[11]. Various clinical studies have established the efficacy of hydrochloride of Berberine in the treatment of oriental sore[15], trachoma[16,17], CHF[18] and Type 2 diabetes mellitus[19,20]. Central nervous system activities of Berberine have been demonstrated in different animal models[12,13,21]. Kulkarni and Dhir (2009) have proposed that Berberine acts as a herbal antidepressant in a dose dependant manner[14].

Based on the ethanobotanical and pharmacological reports presented above, we postulated that the hydrochloride of Berberine could have the potential of a CNS acting drug and could produce behavioral modifications. In order to confirm the findings, we hereby investigated the effects of root extract of *Berberis aristata* plant in mice using behavioral test (righting reflex in mice).

MATERIAL & METHODS

Plant extract

Pure Berberine chloride extract of *Berberis aristata* root was obtained from Hindustan Pharmaceuticals, Amritsar, India. The contents of the product were certified by the company.

Animals

Male & Female Albino mice (20-30gms) were obtained from the Central Animal House of MM Institute of Medical Sciences and Research, Ambala, India. The animals were housed in the groups of 6-8 per cage for a minimum of 5 days prior to pharmacological experiments with free access to standard rodent diet with water and maintained on 12 hr light/ dark cycle and temperature (22°±3°C). The experimental protocol was approved by the Institutional Animal Ethics Committee and CPCSEA. (Ref. n0:106/IAEC/MMIMSR/2011) The minimum number of animals and duration of observation required to obtain consistent data were employed.

Drugs

Diazepam (5mg/ml, Intas Pharmaceuticals Ltd., Ahmadabad, India) was used as a reference drug (positive control) and Thiopentone sodium (1gm, Neon Laboratories Ltd., Mumbai, India) was used to induce hypnosis. Normal Saline acted as a negative control.

Treatment

Berberine chloride was freshly dissolved in suitable amount of distilled water to be acutely administered intraperitonealy (i/p) at 5, 10, 20 mg/kg dose in mice. Diazepam (1mg/ml) and Thiopentone sodium (1mg/ml) were prepared by dissolving in distilled water immediately prior to use and given i/p.

Groups

Mice were divided into 5 groups of 6 animals each and the extract was injected as per the following protocol:

1. Saline (10ml/kg, i/p) as negative control
2. Diazepam (3mg/kg, i/p) as positive control
3. Berberine chloride (5mg/kg, i/p)
4. Berberine chloride (10mg/kg, i/p)
5. Berberine chloride (20mg/kg, i/p)

General Behavioral Observation

Mice were treated i/p with Berberine chloride (5, 10, 20 mg/kg) and placed in observation cage to be observed as described in Modified Irwin Test[22] (continuously from 0-15 min. post dose and discretely at ¼, ½ , 1, 2, 4, 24, 48 hrs. post dose) in simultaneous comparison with vehicle control group. Only the behavioral changes (excitation, sedation and motor coordination) were recorded according to a standardized observation grid.

Thiopentone sodium induced sleeping time

The method employed in this study was described by Vogel[23]. Thiopentone sodium was introduced i/p to all groups at the doses of 40mg/kg body weight. The extract was injected i/p in graded doses (5, 10, 20 mg/kg, i/p) in group 3-5. After 30 min of administration of extract, thiopentone (40 mg/kg, i/p) was given to induce sleep. Group 1 received normal saline as a negative control and group 2 received diazepam (3mg/kg, i/p), 30 min. before thiopentone sodium injection. The onset time of sleep was noted for all the animals. After induction of sleep, mice were placed in the inverted position and when sedation was over, the mice came to normal posture and time was noted. The interval between loss and recovery of righting reflex was used as index of hypnotic effect[23]. The time interval between injection of thiopentone sodium and start of sleep

was recorded as latency time. In the negative and positive control groups, instead of extract, normal saline (10ml/kg, i/p) and diazepam (3mg/kg, i/p) were injected respectively.

Statistical Analysis

The data are expressed as mean±SE. The data were statistically analyzed using Non parametric tests (Kruskal Wallis Test & Mann Whitney Test) to determine significance of differences with respect to the control group. Values of $p<0.05$ were considered as significant. SPSS Inc, Chicago, IL, Version 15.0 software was used for statistical analysis.

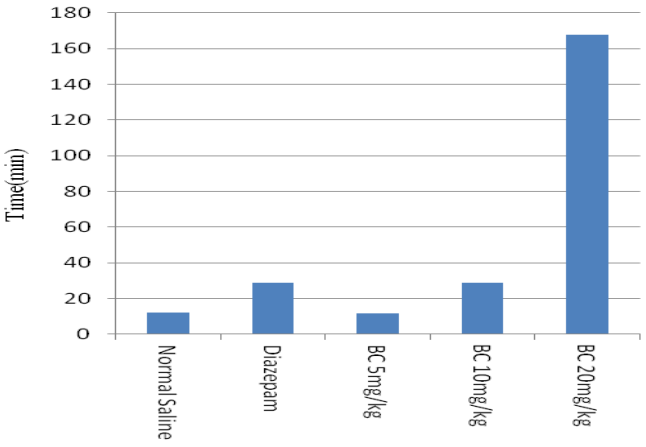
RESULTS

General Pharmacological Observation

Mice were treated with Berberine chloride (5, 10 & 20mg/kg, i/p) and were submitted to general behavioral observation. It was observed that the higher doses(10 & 20 mg/kg) of Berberine chloride produced a significant change in behavior (decreased motility and locomotor activity with motor incoordination) at 10 min, 30 min, 1, 2, 4, 6, 12, 24 and 48 hrs during the observation period, when compared with the control group .

Thiopentone Induced Hypnosis

The acute treatment of mice with 5mg/kg of Berberine chloride increased the sleeping time to 11.50±3.93 min, which was not significantly more than the negative control (12.00±4.00 min, p= 0.675). Those receiving 10 and 20 mg/kg of extract, sleeping time was increase to 28.83±10.24 min and 167.83±52.12 min respectively which was significantly more than the negative control (p=0.003 & 0.004 respectively; fig1). However there was no significant difference between diazepam (28.67±9.81 min) and 10 mg/kg dose (28.83±10.24 min, p=0.054) of the extract. The difference between 10 and 20 mg/kg doses of the extract was highly significant (p=0.004) showing that the higher doses of the extract have a significant CNS depressant action.



Effect of Berberine Chloride in 5, 10, 20 mg/kg doses on thiopentone sodium
ed sleeping time (duration of hypnosis) in mice (data presented as Mean+SE
nice in each group, n=30) (BC= Berberine chloride)

The time interval between the injection of thiopentone sodium and onset of sleep in all groups (Latency time) is presented in fig.2. The extract in the doses of 10 and 20 mg/kg shortened the latency time of sleep to 2.07±0.909 min, p= 0.688 and 0.43±0.318 min, p=0.004 respectively which is lower than that of negative control (2.23±0.173) min. The latency time shortened with 20mg/kg (0.43±0.318 min, p=0.004) of Berberine is comparable to diazepam (0.77±0.127 min, p=0.004). However the dose of 5 and 10 mg/kg of extract decreased latency time to 2.22±0.164 and 2.07±0.909 min respectively but the difference was not significant (p=0.688). Hence, higher doses of extract (20 mg/kg) accelerated the initiation of hypnotic effect of thiopentone sodium comparable to the effect of diazepam (fig 2)

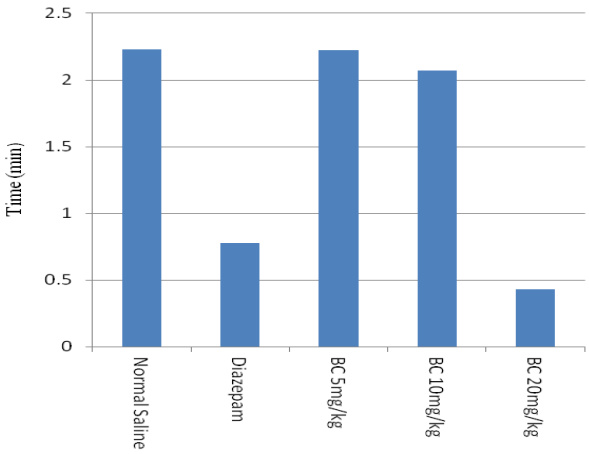


Fig. 2 : Effect of Berberine chloride in 5, 10, 20 mg/kg doses on Latency time of Thiopentone sodium induced sleeping time in mice (Data presented as mean± SE of latency (time of injection to onset of sleep of 6 mice in each group (n=30) (BC- Berberine chloride)

DISCUSSION

The present study investigated the putative central effects of Berberine chloride obtained from the root of *Berberis aristata*, a plant generally used in the Chinese and Ayurvedic medicine as a laxative, ionotropic, antimicrobial and a hepatoprotective remedy. The general pharmacological observation following i/p injection of Berberine chloride was able to promote motor depressant effects in mice. Thus, given acutely at single doses of 10 & 20 mg/kg, Berberine chloride produced significant dose dependant decrease in total motility, locomotor activity, motor incoordination (change in gait) and loss of righting reflex whereas 5mg/kg dose did not show any significant activity.

Several investigators have proposed that this plant possesses central nervous system activity particularly the ability to inhibit mono amine oxidase-A, an enzyme involved in the degradation of NE and 5HT(serotonin)[24]. The same authors have also demonstrated the mechanism of depressant activity of Berberine chloride using different behavioral paradigms. They observed that, at lower doses, this alkaloid did not affect the locomotor activity and barbiturate-induced sleeping time. However, there are studies which have reported the potentiation of barbiturate-induced sleeping at lower doses (4 mg/kg)[25] also. The doses of the drug seem to be crucial to the type of effects obtained by different researchers in various studies. The present study was designed to find out the CNS depressant action of the extract in graded doses of 5, 10 & 20 mg/kg. In agreement with previous reports[24], the results of our study show that the lower doses (5 mg/kg) of the extract did not affect the locomotor activity and thiopentone induced sleeping time where as the dose of 20 mg/kg, significantly reduced the motility and locomotor activity. There was significant motor incoordination and hypnosis was prolonged to 167.83±52.127 min as compared to diazepam 28.67±9.812 min. These findings are indicative of a remarkable sedative effect which was further strengthened by potentiation of thiopentone induced hypnosis after i/p administration of Berberine Chloride. The putative catecholaminergic involvement in the antidepressant like effect of *Berberis aristata* root extract could be suggested.

The alkaloid also bears a benzoisoquinoline alkaloid (morphine) like structure. Various behavioral studies have demonstrated that alkaloids with isoquinoline structure have hypnotic and anticonvulsant properties[26]. It is conceivable that this may be at least partially responsible for hypnotic effect of *Berberis aristata*.

In conclusion, results of our study provide evidence that pure extract of *Berberis aristata* possesses hypnotic property in rodents which was comparable to that of diazepam but the exact mechanism(s) of this effect should be clarified in further studies.

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