

PHARMACOLOGICAL INVESTIGATION OF CASSIA SOPHERA, LINN. VAR. PURPUREA, ROXB.

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SUMMARY: Ethanol extract of seed of Cassia sophera, Linn. var. purpurea, Roxb. was studied for some pharmacological activities in rats.

Eddy's hot plate and Analgesiometer tests were used to assess antinociceptive activity of Cassia sophera. Pentobarbitone narcosis potentiation test was used to evaluate hypnotic and sedative effect, while anticonvulsant activity was evaluated by Maximum electroshock-induced seizure test and Pentylenetetrazol induced seizure test.

Test drug (440 mg/kg) produced significant analgesia, potentiated the pentobarbitone induced sleeping time and exhibited anticonvulsant effect against hind limb tonic extension phase of maximum electroshock-induced seizure test and seizures induced by pentylenetetrazol.

The preliminary screening of seed extract of Cassia sophera, Linn. var. purpurea, Roxb. exhibited analgesic, anticonvulsant effects and potentiated pentobarbitone sleeping time. The ethanol extract of seed of Cassia sophera, Linn. var. purpurea, Roxb. deserve further investigation for elucidation of the mechanisms of action.

Key words: Cassia sophera, anticonvulsant, analgesic, Unani medicine, hypnotic.

INTRODUCTION

Cassia sophera, Linn. (Caesalpiniaceae) known as 'Kasondi' is an important drug of Islamic System of Medicine (Unani Medicine). According to the physicians of Unani medicine, three plants viz., *Cassia occidentalis* Linn. *Cassia sophera* Linn. and *Cassia sophera*, Linn. var. *purpurea*, Roxb. are varieties of 'Kasondi' and are invariably used in similar pathological conditions (1-3). "Kasondi" is described in Unani literature to be repulsive of morbid humours (specially phlegm), resolvent, blood purifier,

carminative, purgative, digestive, diaphoretic (1-4), and reported to be useful in epilepsy, ascites, dyscrasia of liver, skin disorders, piles, jaundice, fever, articular pain and palpitation (1-3, 5).

In ethno botanical literature it is mentioned to be effective in the treatment of pityriasis, psoriasis, asthma, acute bronchitis, cough, diabetes and convulsions of children (6-12). The chemical analysis of the seed of *Cassia sophera*, Linn. revealed the presence of ascorbic acid, dehydroascorbic acid (13) and β -sistosterol (14), but no scientific study is reported on the varietal level of plant. Therefore, in the present study ethanol extract of the seed of *Cassia sophera*, Linn. var. *purpurea*, Roxb. was screened for analgesic and anticonvulsant activity. The

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effect of extract on pentobarbitone sleeping time was also tested.

MATERIALS AND METHODS

Collection and identification of plant material

The seeds of *Cassia sophera*, Linn. var. *purpurea* Roxb., were collected from the plants grown in the herbal garden of Department of Ilmul Advia, Aligarh Muslim University, Aligarh, Uttar Pradesh, India. The seeds were authenticated by Prof. Wazahat Hussain (Botany Department, Aligarh Muslim University, Aligarh, U.P., India). A voucher herbarium specimen was deposited in the Ilmul Advia Department.

Preparation of extract

The collected seeds were powdered and extracted with 70% ethanol in a Soxhlet apparatus for 6 hours. The solvent was evaporated by heating on water bath. The yield of the sample was 17 per cent.

Administration of extract and vehicle

Twenty percent solution of extract dissolved in distilled water (W/V) was administered to the animals of test group in the dose of 440 mg/kg, by a gastric cannula. Distilled water was given to the control group of animals in the dose of 10 ml/kg p.o., in all the tests.

Animals

Studies were carried out on Wistar albino rats of either sex weighing 150-200 g procured from Laboid animal house, Meerut, Uttar Pradesh, India. All experiments were performed in accordance with our institutional Animal Ethics Committee. Animals were used in two groups of six in each experiment except Analgesimeter test, which was performed only on one group. The animals were given commercial diet and tap water *ad libitum*. All the experiments were carried out between 08:00 and 12:00 o'clock at room temperature (25.0°C).

Pharmacological studies

Assessment of analgesic activity

The central analgesic action of the test drug was studied against thermal stimuli using battery of two tests. First, by the method of Eddy's and Leimbach (15) using hot plate, while the second test was done by the method of Davis (16) using Analgesimeter.

Eddy's hot plate test

The initial reaction time of all the animals of control and test groups were recorded by putting them on the Hot Plate maintained at 55.5°C. Licking of paw or jumping was taken as the index of reaction to heat. Thereafter, the control group was administered with distilled water while the test group was treated with test drug.

Post treatment reaction time of each animal was recorded at 20 minutes interval for 120 minutes.

Analgesimeter test

Only one group of six animals was taken. Tail flick response was evoked by placing rat tail over a wire heated electrically. The intensity of heat was adjusted so that the baseline tail flick latency averaged 3-6 seconds in all the animals. Cut off period of 20 seconds was observed to prevent the damage to tail. The initial reaction time of each rat was recorded. The animals were treated with the test drug and post treatment reaction time of each animal was determined at 15 minutes interval for 120 minutes.

Pentobarbitone induced narcosis potentiation test

The control group was treated with distilled water serum physiologic and the test group was administered with the test drug 60 minutes before intraperitoneal administration of pentobarbitone (25 mg/kg). The duration of loss of righting reflex was taken as a measure of sleeping time (17).

Assessment of anticonvulsant activity

Maximum electroshock-induced seizures

The test drug and distilled water were administered to the test and control groups, respectively, 60 minutes before application of electric shock (150 mA for 0.2 seconds) by means of stainless-steel pinna electrodes. The extensor phase of convulsion process was observed (18).

Pentylenetetrazol induced seizure test

The test and control groups were treated with the test drug and distilled water, respectively, 120 minutes before subcutaneous administration of PTZ (80 mg/kg). Both groups were observed for the onset of myoclonic spasm and convulsion up to 30 minutes (19).

Statistical analysis

All the results were statistically analyzed by Student's t-test and expressed as the mean \pm S.E.M.

A value less than 0.05 was considered significant.

RESULTS

Analgesic activity

In Eddy's hot plate test initial reaction times of control and test groups were recorded and they were found 2.96 ± 0.087 and 3.36 ± 0.281 seconds, respectively. Post-treatment reaction time of test group increased to a significant level 3.6 ± 0.19 seconds at 20 minutes interval ($p < 0.05$). The peak effect of test drug was observed at 60 minutes after the treatment and the reaction times of test and control groups at this point of time were recorded 4.6 ± 0.13

Table 1: Analgesic effect of ethanol extract of seed of *Cassia sophera*, Linn. var. *purpurea*, Roxb. (440 mg/kg) by Eddy's hot plate test mean reaction time in seconds.

Time in minutes after drug administration														
	Initial		20		40		60		80		100		120	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Mean	2.96	3.36	3.03	3.6*	3.1	4.26***	3.23	4.6**	3.2	4.16*	3.26	3.9	3.26	3.7
S.E.	0.087	0.281	0.072	0.19	0.061	0.122	0.144	0.13	0.065	0.22	0.161	0.26	0.161	0.312

* p<0.05, ** p<0.01, *** p<0.001, N: 6 albino rats, A : Control group, B : Test group, Values are mean \pm S.E.M.

Table 2: Analgesic effect of ethanol extract of seed of *Cassia sophera*, Linn. var. *purpurea*, Roxb. (440 mg/kg) by analgesiometer test.

Mean reaction time in seconds.									
	Initial	15	30	45	60	75	90	105	120
Ethanol Extract (440 mg/kg)	4.26 \pm 0.167	4.76 \pm 0.136*	5.1 \pm 0.215***	5.56 \pm 0.218***	5.96 \pm 0.185***	6.1 \pm 0.187***	6.1 \pm 0.204***	5.6 \pm 0.156***	5.33 \pm 0.138**

* p<0.05, ** p<0.02, *** p<0.01, **** p<0.001, N: 6 albino rats, Values are mean \pm S.E.M.

seconds (p<0.001) and 3.23 \pm 0.144 seconds, respectively. Although analgesia persisted till the end of testing period but the effect was non significant at 100 and 120 minutes (Table 1).

In Analgesiometer test the initial reaction time was noted 4.26 \pm 0.167 seconds. The significant increase in the reaction time was observed at 15 minutes after drug administration and was 4.76 \pm 0.136 (p<0.05). The peak effect was recorded during 75-90 minutes and the reaction time at 90 minutes interval increased to 6.1 \pm 0.204 seconds (p<0.001) as compared to the initial reaction time in the same group of animals. The analgesia persisted throughout the whole period of testing i.e. till 120 minutes (Table 2).

Hypnotic/sedative activity

In control group the duration of sleep was 122.8 \pm 10.368 minutes. This duration increased to a significant level in test group (p<0.05) and recorded 167.5 \pm 8.401 minutes (Table 3).

Anticonvulsant activity

In Supra maximal electro-shock seizure test it was observed that the test drug produced a significant reduction in the duration of extensor phase which was reduced to 8.1 \pm 0.844 seconds (p<0.05), while in control group this

duration was 11.06 \pm 0.530 seconds (Table 4).

In pentylenetetrazol induced seizure test, the onset of myoclonic spasm and clonic convulsion in control group was observed at 203.3 \pm 15.45 seconds and 228.3 \pm 14.66 seconds after PTZ injection, respectively. All the animals of control group died just after convulsions. The test drug delayed the onset of myoclonic spasm and clonic convulsion to 598.3 \pm 24.2 seconds (p<0.001) and 640.8 \pm 23.67 seconds (p<0.001), respectively (Table 5). All the animals of test group died after 45 minutes of PTZ injection.

DISCUSSION

In Hot plate test, the reaction time of test group increased to a significant level 20 minutes after the treatment (p<0.05). The peak effect was observed at 60 minutes interval (p<0.001). In Analgesiometer test significant analgesic effect appeared at 15 minutes interval and the peak effect was observed at 75 minutes interval. The effect of drug persisted up to 120 minutes. The results of both tests demonstrated more or less similar patterns and some differences in peak effect, probably, because the comparison of reaction time in Analgesiometer test was made with the initial reaction time in the same subjects which is more reliable as it entails least chances of placebo effect, while in hot plate test reaction time was compared with that of the reaction time of control group.

Table 3: Effect of ethanol extract of seed of *Cassia sophera*, linn. var. *purpurea*, Roxb. (440 mg/kg) by pentobarbitone sodium induced narcosis in rats.

Time in minutes		
	Control Group Pent. Sod. + DW	Test Group Pent. Sod. + Ethanol extract
Mean	122.8	167.5*
S.E.	10.368	8.401

* p<0.05, N: 6 albino rats, Values are mean ± S.E.M.

Results of both tests are suggestive of strong analgesic effect in test drug most probably of opioid type as the positive effect against the thermal nociceptive stimuli are indicative of opioid type of analgesic effect (20). Further, the effect starts and attains the peak very early, it therefore, can be used in acute painful conditions. The findings validate the therapeutic use of test drug in different conditions of pain.

In Pentobarbitone narcosis potentiation test, a significant increase in the duration of sleep of test group (p<0.05) was observed, suggesting that the test drug possesses a moderate central depressant effect. Central depressant activity along with strong analgesic effect may complement to each other and thus, may be used in variety of painful and excitatory conditions.

In view of described use of test drug in the management of convulsions of children (12), it was tested for antiepileptic effect. In Maximum electroshock-induced seizure test, the mean duration of extensor phase of test group reduced to significant level as compared to control group (p<0.05). In Pentylene tetrazol induced seizure test, onset of myoclonic spasm and clonic convulsion was delayed in the test group (p<0.001), showing strong antiepileptic effect. The death rate was 100% in both test

Table 4: Anticonvulsant effect of ethanol extract of seed of *Cassia sophera*, Linn. var. *purpurea*, Roxb. (440 mg/kg) by supra maximal electro shock seizure test.

Duration of extensor phase in seconds.		
	Control	Alcoholic extract
Mean	11.06	8.1*
S.E.	0.530	0.844

* p<0.05, N: 6 albino rats, Values are mean ± S.E.M.

and control groups with a difference that in the control group all the animals died just after the onset of clonic convulsions, while, in the test group animals survived up to 45 minutes after PTZ injection. These results further indicates the strong protective effect of test drug against a known epileptic agent. Thus the results of both tests demonstrate very striking and potent antiepileptic activity in test drug that may be useful in both types of epileptic conditions viz., Grand mal and Petit mal epilepsy. Further, by showing antiepileptic activity in spite of having moderate hypnotic and sedative effects as shown in one of the previous tests, it demonstrated specific nature of pharmacological effect of seed of *Cassia sophera*, Linn. var. *purpurea*, Roxb.

Table 5: Effect of ethanol extract of seed of *Cassia sophera*, Linn. var. *purpurea*, Roxb. (440 mg/kg) on seizures induced by pentylenetetrazol in rats.

Group	Onset of myoclonic spasm in sec after PTZ	Onset of clonic convulsion in sec after PTZ
Control (Vehicle + PTZ)	203.3 ± 15.45	228.3 ± 14.66
Alcoholic Extract + PTZ (440 mg/ kg)	598.3 ± 24.2*	640.8 ± 23.67*

* p<0.001, N: 6 albino rats, Values are mean ± S.E.M.

CONCLUSION

'*Kasondi*', although is a popular and investigated plant and commonly used by Unani physicians and others and has been included in many proprietary preparations, but only its sp. *Cassia occidentalis*, Linn. has been subjected for scientific studies. In our study, the seed of *Cassia sophera*, Linn. var. *purpurea*, Roxb., which is frequently used by many physicians and is preferred over *C. occidentalis* in the management of many diseases has been found to possess important pharmacological effects. It includes analgesic, hypnotic and antiepileptic effects. The findings of our study are in consonance with the description of the Unani and ethno botanical literature and it can be used for a wide therapeutic purpose as analgesic, sedative and anticonvulsant agent. The ethanol extract of the seed of *Cassia sophera*, Linn. var. *purpurea*, Roxb. deserve further investigation for detailed elucidation of the mechanisms of action.

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