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Neuropharmacological study of Asokarishta - an Ayurvedic formulation

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SUMMARY

Asokarishta possesses significant analgesic activity. *Adhatoda vasica*, *Nigella sativa* and Asokarista potentiated the pentobarbital sleeping time, where as *Pterocarpus santalinus* antagonized the sleeping time. Excluding *Adhatoda vasica* and *Nigella sativa* rest of them decreased the spontaneous motor activity of the animals. Asokarista exhibited the most powerful antagonist of the amphetamine-induced hyperactivity followed by *Saraca asoca* and *Nymphaea lotus*. But *Nigella sativa* is the only component to increase the amphetamine-induced hyperactivity. The asokarista, *Nymphaea lotus* and *Adhatoda vasica* showed significant depressant effect on hole cross test. Asokarista, *Adhatoda vasica*, *Nigella sativa*, *Nymphaea lotus* and *Saraca asoca* also showed reduced movements in open field experiment. The asokarista and its major components exhibited depressant effect on ambulation and head dipping on hole-board test. Climbing out experiment also support the previous findings.

Key words: Asokarishta, *Saraca asoca*, *Nigella sativa*, *Nymphaea lotus*, *Adhatoda vasica* , *Pterocarpus santalinus*, Neuropharmacological

INTRODUCTION

In the Ayurvedic system of medicine, the preparation Asokarishta is indicated as a drug of choice for the treatment of dysmenorrhea and menorrhagia. Dysmenorrhea which explained as painful menstruation is always associated with a lot of distressful central nervous system symptoms. To observe the neuropharmacological effect of the preparation Asokarishta and five of its major components, Ashokchal (*Saraca asoca*), Krishnajira (*Nigella sativa*), Raktautpal (*Nymphaea lotus*), Raktachandan (*Pterocarpus santalinus*), and Basak (*Adhatoda vasica*) were evaluated by a battery of CNS experiments, which are designed to show the possible neuropharmacological effect.

According to Bangladesh National Formulary of Ayurvedic Medicine (Anonymus, 1992), the preparation of Asokarishta consists of components listed in Table 1.

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MATERIALS AND METHODS.

Animals

The experimental animals employed throughout the study were Swiss-Webster mice (body wt. 20-25 gm). The food supplied to the animals was prepared according to the balance diet formula for laboratory rats and mice developed by the Animals Resource of BCSIR, Dhaka. The animals were provided with tap water. All animals were given 10 days rests before being employed in different tests, to adjust themselves with the new environment.

Preparation and feeding of the drug

The preparation Asokarishta was administered to the test animals according to the body weight. In case of individual five components of Asokarishta (ASK), the powder of the components was dispensed

as kwath, which was prepared by adding 16 times (v/w) distilled water with a known weight of plant powder and was mixed thoroughly. After adding water, the mixture was boiled till the volume was reduced to one-fourth of the initial volume. The mixture was filtered and the filtrate was collected. This filtrate was called kwath and it was administered either orally or intraperitoneally (i.p.), according to the test requirements. The dose of the kwath was determined according to the body weight of the test animals. The animals of the control group were simultaneously administered with the equal volume of 0.9% normal saline.

Dose and route of administration of ASK and its individual components

For the study of Asokarista and its individual components the doses used were 10 ml of kwath/kg body weight orally.

Hot-plate test

The analgesic study was conducted by the "Hot Plate" method, described by Woolfe *et al.* (1944) and Wood (1985). Hot plate was maintained at a constant temperature of 55±0.5°C. Each mouse was placed on the hot surface and the time of response to this thermal stimuli, indicated by the licking of hind and/or fore paws or by kicking of the legs or by trying to jump-out, was recorded.

Spontaneous motor activity in mice

This experiment was carried out by modified version of the "Sand-Displacement" method developed by Siegmund and Wolf (1952). The displaced brick chips, through the wire nettings due to the spontaneous motor activity of the animals, were recorded with 5 minutes interval for a period of one hour. The recordings were compared with that of the control animals.

Amphetamine induced hyperactivity

In this experiment, the "Sand-Displacement" method was employed (Siegmund and Wolf, 1952). Amphetamine was administered at a stimulant dose of 4 mg/kg body weight (Vane, 1961).

Hole-cross test

This experiment was carried out by the method of Takagi *et al.* (1971). A steel partition was fixed at the middle of a cage 30×20×14 cm in size. A hole of 3 cm in diameter was made at the height of 7.5 cm in the center of the plate. The number of passages of a mouse through that hole from one end of the cage to another was recorded for a period of two minutes at 0, 30, 60, 120 and 240 minutes. Similar recordings were made for the control animals.

Table 1. Composition of the drug Asokarishta

	Name of the components	Family	Parts used	Amount used
1	*Saraca asoca* (Roxb) De Wilde.	Caesalpiniaceae	Bark	10 kg
2	*Woodfordia fruticosa* Kurz.	Lythraceae	Flower	1.6 kg
3	*Nigella sativa* Linn.	Ranunculaceae	Seed	100 gm
4	*Cyperus rotundus* Linn.	Graminae	Rhizome	100 gm
5	*Zingiber officinale* Rosc.	Zingiberaceae	Rhizome	100 gm
6	*Berberis aristata* DC.	Berberidaceae	Wood	100 gm
7	*Nymphaea lotus* Hook. f. and Thoms.	Nymphaeaceae	Root/Flower	100 gm
8	*Terminalia chebula* Retz.	Combretaceae	Fruit rind	100 gm
9	*Terminalia bellirica* Roxb.	Combretaceae	Fruit rind	100 gm
10	*Phyllathus emblica* Linn.	Euphorbiaceae	Fruit rind	100 gm
11	*Mangifera indica* Linn.	Anacardiaceae	Seed kernel	100 gm
12	*Adhatoda vasica* Nees.	Acanthaceae	Bark	100 gm
13	*Pterocarpus santalinus* F.	Papilionaceae	Wood	100 gm
14	Molasses	-	-	20 kg
15	Water	-	-	100 lt.

Open-field test

This experiment was carried out according to the method developed by Gupta *et al.* (1971). The floor of an open field of half square meter was divided into a series of squares, each alternatively colored black and white. The Apparatus had a wall of 40 cm. The number of squares traveled by the animals, was recorded for a period of two minutes at 0, 30, 60, 120 and 240 minutes. Similar recordings were made for the control animals.

Hole-board test

For this experiment, the method deviced by Nakama *et al.,* (1972) was followed. In a flat surface of half square meter, 16 holes, each 3 cm in diameter, were uniformly distributed. The number of holes explored (head dipping), holes passed (ambulation) and defecation was recorded for each animal for a period of 2 minutes at 0, 30, 60, 120 and 240 minutes. Similar recordings were made for the control animals.

Climbing-out test

This experiment was carried out by the method of Sandberg (1957). The animals were put in a cage with dimension of 60×50×35 cm, and having dark walls. They were supplied with a ladder and the time taken to climb out of the cage was recorded for a maximum period of 10 min.

Pentobarbital narcosis test

Pentobarbital sleeping time was carried out according to the method deviced by Tedeschi and Tedeschi (1968). The drug was administered intraperitoneally 30 min before the administration of pentobarbital to a group of 8 mice. Pentobarbital was administered at a dose of 45 mg / kg. The time of administration of pentobarbital in both the control and drug treated animals were recorded and the animals were observed for the onset and the duration of sleep, as evidenced by the observation of the loss of righthing reflex until the animal woke up.

Statistical analysis

Data obtained from the experiments are expressed as mean and standard error of the mean (S.E.M.). Unpaired t-tests were performed by computer software SPSS release 6.0 for Windows™, to test the level of significance. Probability (p) value of 0.05 or less ($p<0.05$) was considered as significant. In this paper $p<0.05$, $p<0.01$, $p<0.001$ are represented by a single (*), double (**) and triple (***) asterisk(s).

RESULTS

In the Ayurvedic system of medicine, the preparation of Asokarishta (ASK) is indicated as a drug of choice for the treatment of dysmenorrhea and menorrhagia. Dysmenorrhea which is explained as painful menstruation associated with a lot of distressful central nervous system symptoms. In this study the preparation of Asokarishta and five of its major components of Ashokchal (*Saraca asoca*, ACL), Krishnajira (*Nigella sativa*, KSJ), Raktautpal (*Nymphaea lotus*, RPL), Raktachandan (*Pterocarpus santalinus*, RCN), and Basak (*Adhatoda vasica*, BSK), were evaluated by a battery of neuropharmacological experiments.

Effect on hot-plate

The preparation of ASK shows highly significant analgesic effect ($p<0.001$). The analgesic effect also persisted for the duration of the entire study. Whereas the RCN showed an increased sensitivity to this test (Table 2).

Effect on pentobarbital narcosis test

As many centrally active drug work on the cerebral cortex and thus produce their actions (Bowman and Rand, 1980), the chances of ASK and its components having effect on the cerebral cortex were evaluated by pentobarbital narcosis test.

The experimental data showed that the onset of sleeping time of the animal was enhanced by the test drug and some of its components in the following order:

BSK>>ACL>ASK

The duration of sleeping time was also increased in the following order:

BSK>KSJ>ASK

On the contrary RCN shortened the duration of sleeping time. The results of the pentobarbital narcosis experiment indicate the possibility of ASK

Table 2. Effect of ASK and its individual components on pain perception test

Groups	Pain Perception Test (sec) Mean±SEM (p value)				
	0 min	30 min	60 min	120 min	240 min
CON (n=18)	13.12±1.117	13.32±2.02	25.16±1.91	15.25±1.50	12.72±0.98
ASK (n=6)	10.28±1.26 (0.206)	30.91±5.18 (0.001)***	28.58±4.1 (0.003)**	23.58±2.86 (0.013)***	24.25±3.96 (0.033)*
ACL (n=6)	14.66±1.23 (0.485)	6.4±0.55 (0.004)**	13.33±3.7 (0.648)	3.5±4.52 (0.362)	15.16±3.66 (0.378)
KSJ (n=6)	20.50±1.204 (0.217)	32.83±3.604 (0.407)	28.33±3.57 (0.003)**	4.66±1.67 (0.03)*	16.66±2.47 (0.085)
RPL (n=6)	28.33±1.67 (0.000)***	12.33±2.12 (0.793)	11.66±3.07 (0.364)	10.16±3.01 (0.116)	8.83±4.27 (0.196)
BSK (n=6)	27.16±2.89 (0.003)**	16.16±2.89 (0.474)	27.83±6.14 (0.015)*	37.5±7.5 (0.030)*	32.5±8.24 (0.062)
RCN (n=6)	16.0±1.41 (0.206)	12.67±2.81 (0.866)	8.0±1.23 (0.048)*	7.33±1.38 (0.008)**	16.0±3.72 (0.231)

Table 3. Effect of ASK and its individual components on pentobarbital-induced sleeping time

Groups	Onset of sleeping (sec) Mean±SEM (p value)	Duration of sleeping (sec) Mean±SEM (p value)
CON (n=30)	297.40±33.72	3227.33±268.51
ASK (n=12)	235.0±23.88(0.393)	4080.0±299.72(0.075)
ACL (n=6)	200.0±12.64(0.01)**	3210.0±219.49(0.978)
KSJ (n=6)	315.0±13.73(0.000)***	4460.0±344.70(0.014)*
RPL (n=6)	308.58±97.29(0.891)	3536.0±321.53(0.517)
BSK (n=6)	120.0±0.0(0.000)***	4530.0±626.37(0.057)
RCN (n=6)	319.83±34.21(0.70)	2526.83±187.7(0.039)*

and its components having actions on the cerebral cortex (Table 3).

Effect on spontaneous motor activity test

The outcome of the experiment showed that ASK and its five components decreased the spontaneous motor activity of the animals. The magnitude of locomotor retarding effect of the test drugs can be exhibited by the following series:

ACL>ASK>RCN>RPL>KSJ>BSK

The results of the experiment are graphically presented by Fig. 1.

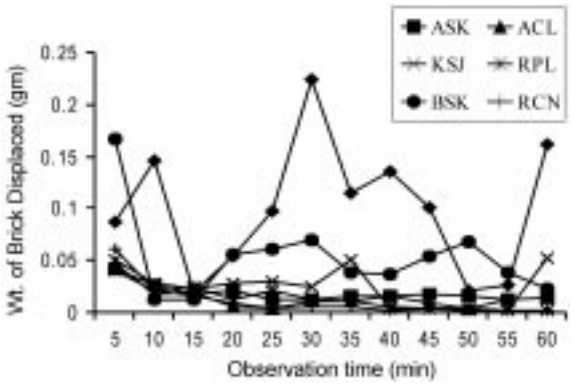

Fig. 1. Effect of ASK and its individual components on spontaneous motor activity.

Effect on amphetamine-induced hyperactivity test

In this experiment the test drug was administered in two different dose levels. The experimental findings at the lower dose level (10 ml/kg body wt.) showed that amphetamine induced hyperactivity of the treated animals was decreased by the components in the following order:

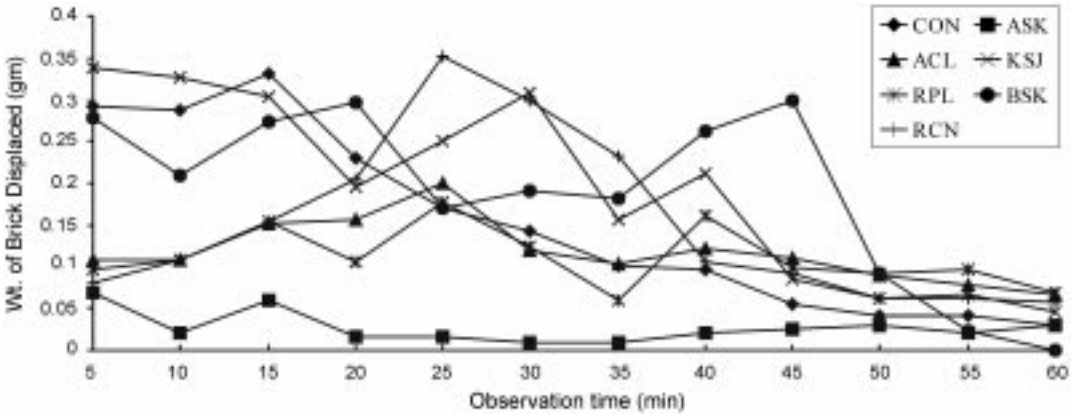

Fig. 2. Effect of ASK and its individual components (dose=10 ml/kg bd. wt.) on amphetamine-induced hyperactivity.

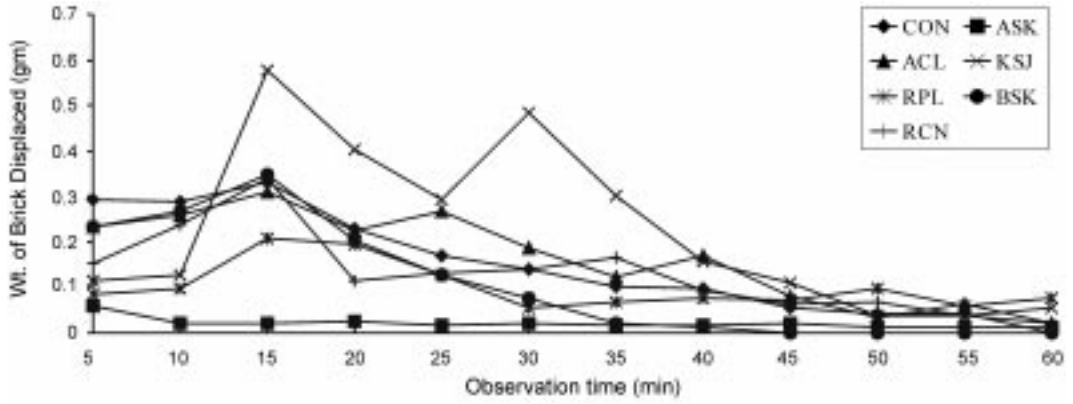

Fig. 3. Effect of ASK and its individual components (dose=40 ml/kg bd. wt.) on amphetamine induced hyperactivity.

ASK>ACL>RPL>RCN>BSK

It was observed that ASK, ACL and RPL decreased the amphetamine-induced hyperactivity significantly for up to 30 min. But RCN showed decreased hyperactivity between 25 and 35 min of study. KSJ at lower dose also showed increased activity between 15 and 30 min interval (Fig. 2).

The experimental findings (Fig. 3) at the higher dose level (40 ml/kg body wt.) of the test drug indicate that, in such a dose, the amphetamine-induced hyperactivity was also decreased in the following order.

ASK>RPL>ACL>BSK>RCN

But KSJ in this higher dose also increases the hyperactivity of the treated animals in this experiment.

Effect on hole cross test

The propulsive movement of the treated animals was evaluated by the hole cross test. The experimental data indicate a decreased level of propulsive movement of the treated animals by ASK and its components and the magnitude of the effect can be shown by the following series:

ASK>RPL>BSK>ACL>RCN>KSJ

The depressant effect of ASK, RPL and BSK are significant (Fig. 4).

Effect on open field test

The test is designed in such a manner so that the test animal is not assigned to perform any specific task. It has been reported that in the absence of any specific task to perform, the behavior of a given animal tend to maintain that inner activation level

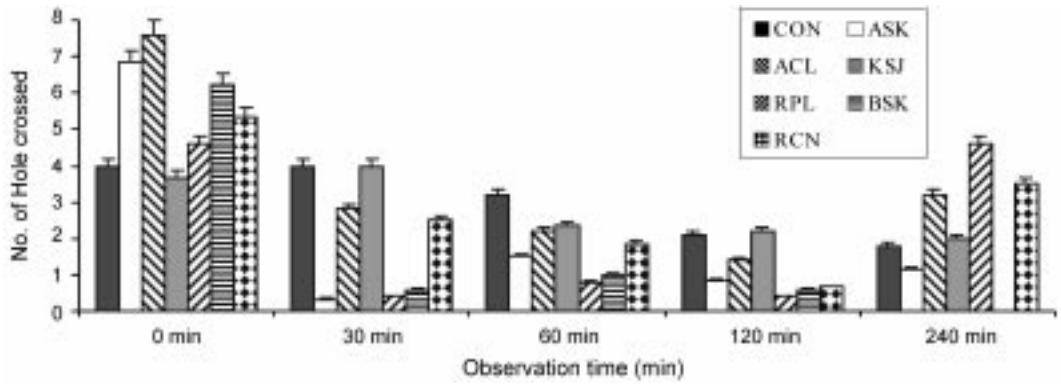

Fig. 4. Effect of ASK and its individual components on hole cross.

Table 4. Effect of ASK and its individual components on open field

Group	No. of Movement, Mean±SEM (p value)				
	0 min	30 min	60 min	120 min	240 min
CON (n=18)	95.67±7.62	74.44±8.28	71.83±10.22	70.83±9.46	62.50±9.92
ASK (n=6)	114.17±22.86 (0.015)*	12.50±4.45 (0.000)***	6.0±1.98 (0.000)***	6.67±2.26 (0.000)***	4.0±2.27 (0.000)***
ACL (n=6)	110.83±17.68 (0..37)	74.44±8.28 (0.047)*	21.33±8.14 (0.012)*	11.83±2.76 (0.000)***	19.0±7.86 (0.024)*
KSJ (n=6)	140.5±18.09 (0.013)*	19.0±5.83 (0.001)***	23.0±10.14 (0.017)*	7.33±2.91 (0.000)***	16.33±4.36 (0.000)***
RPL (n=6)	94.67±14.55 (0.949)	26.50±6.25 (0.004)**	5.67±2.19 (0.000)***	13.17±4.76 (0.002)**	62.50±9.92 (0.000)***
BSK (n=6)	111.5±26.94 (0.593)	41.16±22.25 (0.094)	23.50±9.96 (0.017)*	9.66±3.16 (0.000)***	6.0±1.83 (0.000)***
RCN (n=6)	103.33±16.26 (0.639)	76.17±6.65 (0.910)	68.67±17.38 (0.878)	57.67±14.0 (0.480)	68.0±10.62 (0.768)

that is at times, in consistent with the actual level of activation of the animal. ASK and its components except RCN showed significant depressant effect in this experiment (Table 4). The magnitude of the depressant effect is the following order

ASK>BSK>KSJ>RPL>ACL

Effect on hole board test

A similar exploratory head dipping retarding effect was observed by the experimental data. The severity of the head dipping retarding effect, in decreasing order can be shown (Fig. 5) by the following series

RPL>ASK>ACL>BSK>KJS>RCN

The data on defecation suggest that the degree of emotional defecation was reduced in the treated animals in comparison to those of the control animals (Fig. 6). The magnitude of effect can be illustrated in the following order

RPL>KSJ>RCN>BSK>ASK>ACL

Data of ambulation also showed significant decreased movements in the study (Fig. 7). The magnitude of effect was as follows

ASK>RPL>KSJ>ACL

The BSK and RCN had no effect on this ambulation study of hole board test.

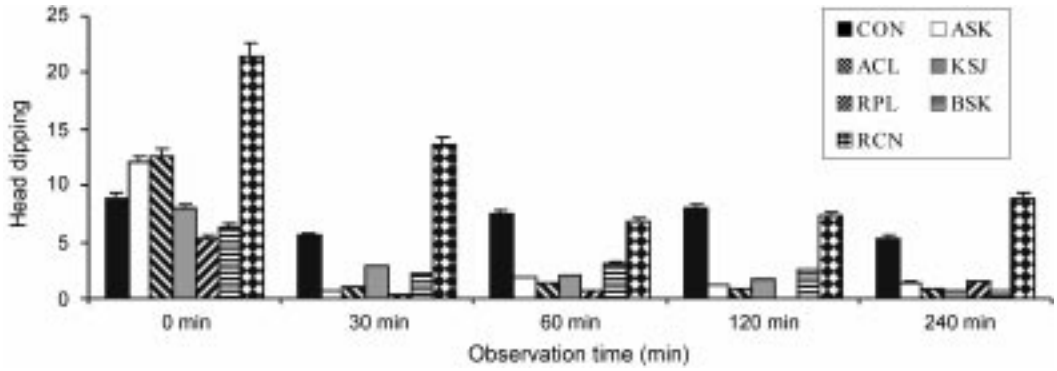

Fig. 5. Effect of ASK and its individual components on hole board (head dipping).

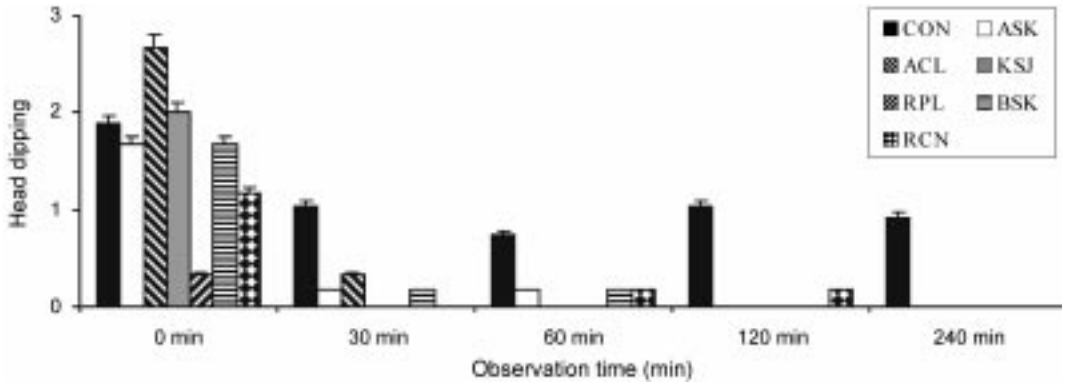

Fig. 6. Effect of ASK and its individual components on hole board (defecation).

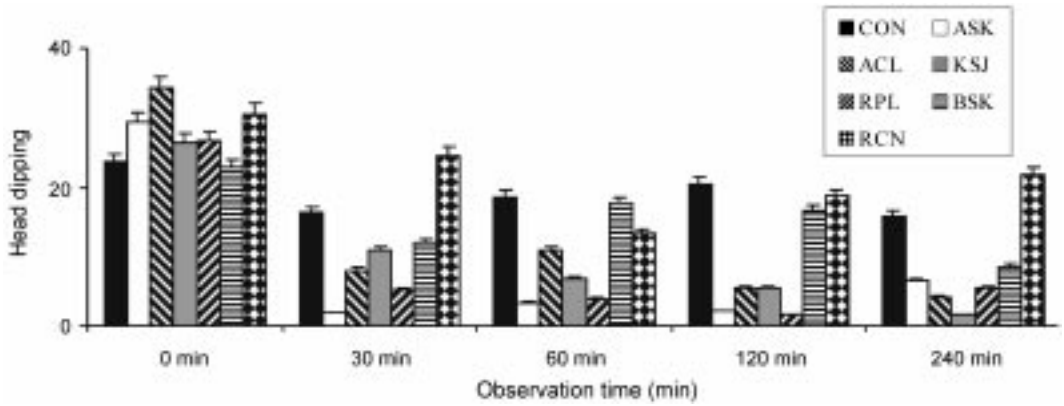

Fig. 7. Effect of ASK and its individual components on hole board (ambulation).

Effect on climbing out test

This experiment checks the effect of the test drug and its components on general behavior of the treated animals by a model to perform a specific task. In corroboration to the previous findings, the animals inquisitiveness was suppressed by the components of the drug, in this experiment too (Fig. 8), in the following order

KSJ>ASK>ACL>BSK>RCN>RPL

DISCUSSIONS

Asokarishta is a classical Ayurvedic preparation, which is used for the treatment of dysmenorrhoea and menorrhagia. The major of its components are *Saraca asoca* (Syn. *Saraca indica* Linn.), *Nigella sativa,*

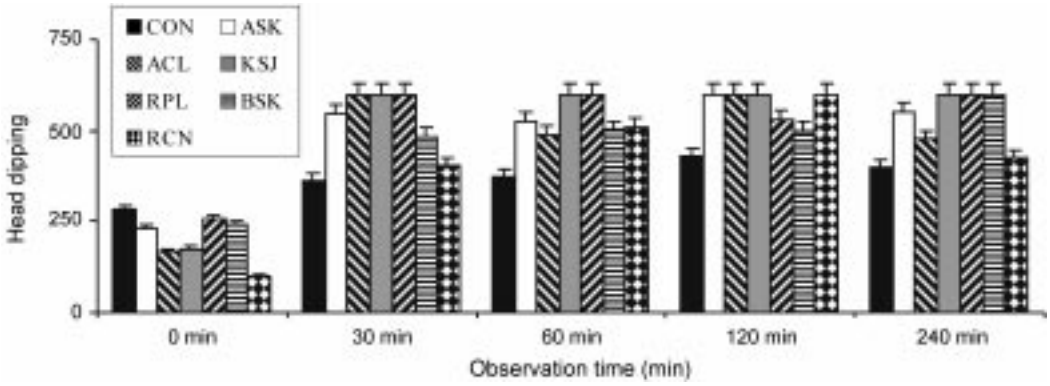

Fig. 8. Effect of ASK and its individual components on climbing out.

Nymphaea lotus, Pterocarpus santalinus and *Adhatoda vasica*. From these studies it has been found that, Asokarishta may be antagonize mild to moderately the morphine receptors and possesses some central nervous system depressant activities. These CNS effects may due to the presence of *Saraca indica* Linn. (Ashoka), *Nigella sativa* Linn. (Kalijira), and *Nymphaea lotus* (Raktoutpal). These are commonly CNS depressant and in these studies it was found that these three component of the formulation Asokarishta individually exhibited mild to moderate CNS depressant activities, though there was no previous report supporting the CNS depressant activities of *S. indica* Linn.

Satyavati *et al.* (1970a and 1970b) reported that a pure phenolic glycoside (P2) from *Saraca indica* Linn. (Ashoka) showed oxytocic and uterine activities. This may explain its effects on the female organs, especially the uterus. In a study it was found that ten cases of metromenorrhagia were given 2 ml of injection of Ashoka (i.m.) bi-weekly for 3 months. All the patients responded extremely well and the menstrual flow was regularized without any complication (Sedani, 1990). It was also suggested that, the Ashoka may be useful in all cases of uterine bleeding where ergot is indicated (Mukherji, 1970).

The components of *Nigella sativa* proved as very good analgesic, both narcotic and non-narcotic system, anti-inflammatory agent in many previous reports. *N. sativa* oil and thymoquinone produce antinociceptive effects through indirect activation of the supraspinal mu(1)- and kappa-opioid receptor subtypes (Abdel-Fattah, 2000). The crude fixed oil and pure thymoquinone both inhibited the cyclooxygenase and 5-lipoxygenase pathways of arachidonate metabolism in rat peritoneal leukocytes stimulated with calcium ionophore A23187, as shown by dose-dependent inhibition of thromboxane B_2 and leukotriene B_4, respectively. Thymoquinone was very potent, with approximate IC_{50} values against 5-lipoxygenase and cyclo-oxygenase of <1 μgm/ml and 3.5 μgm/ml, respectively. Both substances also inhibited non-enzymatic peroxidation in ox brain phospholipid liposomes, but thymoquinone was about ten times more potent. However, the inhibition of eicosanoid generation and lipid peroxidation by the fixed oil of *N. sativa* is greater than is expected from its content of thymoquinone (ca. 0.2% w/v), and it is possible that other components such as the unusual C20:2 unsaturated fatty acids may contribute also to its anti-eicosanoid and antioxidant activity. These pharmacological properties of the oil support the traditional use of *N. sativa* and its derived products as a treatment for rheumatism and related inflammatory diseases (Houghton, 1995). The aqueous extracts of the seeds of the *N. sativa* also exhibited mild to moderate analgesic and anti-inflammatory activities on laboratory animals (Khan, 1999).

The crude extract of *Nigella sativa* seeds exhibits spasmolytic and bronchodilator activities mediated possibly through calcium channel blockade (Gilani *et al.*, 2001). The methanol soluble portion of black cumin oil, which is prepared by compression of seeds of *N. sativa* Linn., showed inhibitory effects on arachidonic acid -induced platelet aggregation and blood coagulation. a new compound 2-(2-

methoxypropyl)-5-methyl-1,4-benzenediol from the methanol soluble part and two known compounds, thymol, carvacrol, having very strong inhibitory activity (Enomoto *et al.*, 2001).

Nymphaea lotus (Raktoutpal) does not have any such kind of previous report. Mehta in 1979 reported that, the 90% ethanolic extract of the heartwood and bark when administered showed no analgesic activity. But it exhibited appreciable protection against carrageenin-induced edema in rat paw at dose of 1.0 to 2.0 gm / kg. It also showed CNS depressant and tranquilizing activities (at 1.0 gm / kg protection about 50%), anticonvulsant action against electro-shock convulsions, on experimental animals (Mehta, 1979). There was no previous report for analgesic and CNS activities of the extracts of *Adhatoda vasica.*

In the Ayurvedic medical system the Asokarishta is used for the treatment of dysmenorrhoea and menorrhagia, more animal and clinical trials should be done to pin-point the active plant. Then the active pure compound of that plant should be isolated and recognized.

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