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Research Article

AN EXPLORATORY STUDY ON THE CENTRAL NERVOUS SYSTEM EFFECTS OF *KOPSIA FRUTICOSA* ON LABORATORY ANIMALS

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ABSTRACT

Drugs bearing indole alkaloids are known to produce important pharmacological effects. *Kopsia fruticosa* (KF), member of thee Genus Kopsia containing four indole alkaloids possesses central nervous system (CNS) pharmacological effects. This study was conducted to explore the CNS effects of KF in rodents.

Materials and Methods: The propylene glycolic solution (KF1) and aqueous solution (KF2) were prepared from the dried extract of the leaves of KF. Evaluation of hexobarbitone induced sleeping time in mice, analgesic effect by Tail flick response on thermal stimulus in rats, locomotor activity by Spontaneous motor activity and rotarod method in rats, sedative ataxic score by inclined plane method in mice and behavioural activity by Gross behavioural effects, Conditional Avoidance response, and cataleptic effects in rats were performed at 2, 4, 8,16 mg/kg of body weight respectively.

Results: KF1 reduced the spontaneous locomotor activity and exploratory behaviour in mice ($p<0.001$-0.01) more than KF2. KF1 in all doses potentiated the hypnotic dose ($p<0.001$) of hexobarbitone and sub-hypnotic dose (upto 80% potentiation) of hexabarbitone as compared to only 40% potentiation by KF2. Anti-nociception effect was found to be absent at all doses. KF1 possesses moderate sedative ataxic effect ($p<0.001$) and KF2 at 8mg/kg b.w. revealed mild effect ($p<0.05$) when compared to pentobarbitone. KF1 induced a moderate degree of catalepsy and potentiated cataleptic and sub-cataleptic doses of haloperidol at all doses ($p<0.001$).

Conclusion: The present study has revealed that the propylene glycolic extract of the leaves *Kopsia fruticosa* possesses profound action on the CNS thereby paving the way to further research.

KEYWORDS: *Kopsia fruticosa*, hexobarbitone induced sleeping time, sedative atyaxic score, catalepsy.

INTRODUCTION

Drugs bearing indole alkaloids are known to produce important pharmacological effects. Genus Kopsia belonging to Apocynaceae family contained about 34 species and most of the plants under this genus were reported to possess indole alkaloids. *Kopsia fruticosa*, member of this genus contains four indole alkaloids (Fig. 1& 2) whose pharmacological effects have not been extensively studied. It has been reported that most of the indigenous plant having indole alkaloids exhibit varieties of pharmacological actions like hypotension, central nervous system depression, local anaesthesia, dilatation of both peripheral and coronary vessels and antileukemic effect etc.[1]

Hence it was conceived that the compounds of *Kopsia fruticosa* (KF) may produce some interesting pharmacological actions especially on the central nervous system. The crude extract was prepared from the dried leaves in laboratory and detailed pharmacological studies were carried out. The chief objective of this study was to evaluate the possible effects of KF on the central nervous system in laboratory animals.

MATERIALS AND METHODS

Test formulations and animals used for the study

The leaves of *Kopsia fruticosa* were provided by the Central Council for Research in Ayurvedic Sciences (CCRS), Kolkata, West Bengal. Solutions for the present study were prepared from the dried total extract of leaves of KF using the Soxhlet apparatus and the propylene glycolic solution of KF was designated as KF1 while the aqueous solution of KF designated as KF2. The animals used for this study were recruited from Indian Institute of Chemical Biology (IICB), Kolkata, West Bengal.

Ethics Clearance

The present work was MD Thesis dissertation work of SM during 1992-94 at the department of Pharmacology of Institute of Post-Graduate Education and Research (IPGMER) and Seth Sukhlal Karnani Memorial Hospital (SSKM), Kolkata. At that time Institutional Animal Ethics Committee (IAEC) was not in vogue and the permission to initiate and continue this research work was granted by the guide Professor Dipankar Bhattacharyya, and head of the department of Pharmacology.

Effect on hexobarbitone induced sleeping time in mice

The sleep evaluation method was based on potentiation of hexobarbital-induced sleeping time.[2] The animals were administered a single dose of the vehicle, diazepam, and the extracts by intraperitoneal (i.p.) route. Hexobarbitone Sodium (100 mg/kg, i.p.) was injected 30 minutes after administration of the test drug and vehicle in all groups for screening of centrally acting compounds.[2] The time elapsed between loss and recovery of the righting reflex was noted and taken as sleeping time. This reflex was considered positive when the animal placed on its side recovers from this position within one minute. It was considered lost when the recovery requires longer period. This time has been expressed in minutes. Sleeping time was expressed as Mean ± SEM.

The compounds KF1 and KF2 were studied on hexobarbitone induced hypnosis in mice following the methods of Kopera and Armitage[3] and UK Seth[4] with some modifications. There were eight groups of six albino mice each. KF1 and KF2 were administered to six groups of animals in doses of 4 mg. 8 mg and 16 mg/kg body weight i.p. respectively. In all the groups of animals, drugs were administered 15 minutes before the administration of hexobarbitone sodium (100 mg/kg. body wt.) i.p. Next we tried the hypnotic prolongation effect of KF1 and KF2 and subhypnotic dose of hexobarbitone i.e. 50 mg/kg. body weight, i.p. The duration of sleep was taken to be the time between loss of righting reflex and the re-appearance of the same. The experiment was carried out at room temperature and the mean sleeping time of each of the control and the drug treated groups were recorded accordingly.

Study on Analgesic effect - Tail flick response of rat on thermal stimulus

Rat tail flick response to thermal stimulus was utilized for the measurement of analgesic activity. The use of radiant heat as a thermal stimulus for the measurement of strong analgesics was employed by D' Amour and Smith (1941)[5], Guzral & Khanna (1957)[6] using electrically heated nichrome wire. A "Techno analgesiometer "served this purpose and was employed for the analgesic tests of the compounds. Wistar Albino rats of either sex weighing between 150 to 250 gm were assigned to eight groups, each comprising of five animals were used for the test. One group of the rats served as control and was treated with normal saline 0.5 ml i.p. The other six groups were treated with KF1 and KF2 in doses of 4, 8 and 16 mg/kg body wt. i.p. 15 minutes before the administration of morphine respectively. Morphine (Ms), as a standard analgesic agent was used in another group at the dose of 10 mg/kg. i.p. and the potentiating effect of KF1 and KF2 on subanalgesic dose of morphine i.e. 2 mg/kg. body wt i.p. was then studied. At first a control study with rats treated with normal saline was made. Then rats treated with test drugs KF1, KF2 and morphine were put on analgesiometer at the rate of one animal at a time. The time taken to flick the tail in response to radiant heat was considered as the end point and taken as the withdrawal time 30 seconds was taken as the cut off time and the observations were noted and then tabulated.

Studies on the locomotor activity

Spontaneous motor activity

The method followed to determine the effect of KF1 and KF2 on spontaneous activity of mice (15 to 20 g. of body weight) was essentially that of Szymanski (1914)[7] with some modification. Light wiremesh cages measuring about 10 cm x 10 cm were suspended from a hook by a light rubber string. A thread was tied at the bottom of the cage while the other end was tied to a frontal lever through pulleys. Movements of the animal kept in cage resulted in oscillation of the cages which was recorded on a smoked slowly moving drum with the help of frontal writing lever. Three mice were kept one at a time in the three cages and their movements were recorded and the animals which were found to be docile were discarded. Three groups of mice containing five mice in each group were thus selected in doses of 2.5, 5 and 10 mg/kg body wt. of KF1 and KF2 respectively and the other group were treated with the drug KF2 in the same doses like KF1. Observation was made for 4 hours after the administration of drugs. Increase or diminution in the spontaneous motor activity in mice was adjusted by increase or diminution in the amplitude of recording on the smoked drum.

Rota rod method with rats

The present study was carried out with rats weighing from 150 to 200 g were placed on a horizontal scrapped iron rod, having a diameter of 2 cm. and rotating at the rate of 10 revolutions per minute. Circular sections were used to divide the linear space of the rod in 6 lengths, so that 6 rats could be tested at a time and they did not interact with each other. The rod was placed at least 15 inches above the table top to prevent the animals from spontaneously jumping off the rod[8]. Five groups of such animals, each group containing 5 rats were selected. The first group was injected intraperitoneally with normal saline (control), and 3rd group were injected i.p. with propylene glycol 0.5 ml. while the other two groups were injected i.p. with glycolic solution of *K. fruticosa* (total extract) in doses 2.5 and 5 mg/kg of body weight respectively. Each group was then placed on the rod after an interval of 30 minutes. An animal falling more than once to remain on the rod for 3 minutes the test was considered to be positive. The number of falls during the 3 minutes period were noted and the mean of the number of falls against each dose was calculated and tabulated.

Evaluation of sedative ataxic score (inclined plane method with mice)

The inclined screen test is suitable to rapid and fairly accurate estimates of sedative activity and is simple to carry out [9]. In this study the sedative ataxic score of *K. fruticosa* (KF1) were carried out by following the method of Bhargave and Gupta (1977)[10]. Seven groups of six mice screened beforehand were used for evaluation of the sedative ataxic effect of *K. fruticosa* were treated with hexobarbitone to compare the sedative ataxic effect of the plant extract with a standard hypnotic drug. Doses of the drug KF1, KF2 and hexobarbitone selected were 4 mg and 8 mg/ kg body wt. respectively. One of group of mice was treated with normal saline 0.5 ml i.p. In all the cases drugs were injected i.p. 15 minutes before starting the

experiment. Sedative ataxic score was then determined by following the method of Tislow (1966)[11] and it was graded as slight (score of 1-2) moderate (score of 3-4) or marked (score of 5). The scores obtained by observing the activity of five animals in each group were determined and tabulated in Table - 1.

Table 1: Detection of sedative ataxic score

Score	Toleration of side or back position	Activity in vertical pole	Activity on inclined pole
0	No	Grasps pole & walks down	Moves actively on screen
1	No	Grasps pole & slide down	Moves actively on screen
2	No	Grasps pole & slide down	Cannot move at all only sluggishly
3	No	Cannot grasp pole	Cannot move at all only sluggishly
4	No	Cannot grasp pole	Cannot hold on to the screen

Effects of the behavioural activity

Gross behavioural effects

Mice in five groups of 6 animals in each were taken and the four groups were treated i.p. with KF1 and KF2 in doses of 4 mg/kg body wt and 8 mg/kg body wt respectively. The fifth group received 1 ml of NS i.p. The changes in the behavioural attitude was noted, if any, for a period of 2 hours in intervals of 30 minutes each.

Conditional Avoidance response in rats

To study the effect of *Kopsia fruticosa* on conditional avoidance response, the technique of " Jumping box " as described by Warner (1932) with some modification by Piala et al., (1959)[12] was used. Thirteen groups of rats containing 5 animals each were selected for the experiment. One group served as control and was treated with normal saline 0.5 ml i.p. the other twelve groups were divided into four broad groups so that each broad group contained three groups of 5 animals each. Those broad groups were treated with hexobarbitone, KF1, KF2, and chlorpromazine. KF1 and KF2 were given in doses of 2.5, 5 and 10 mg/kg body wt. respectively to each three groups of the four broad group of animals. Drugs were administered by i.p. route 15 minutes before start of the experiment. Response of the animals in response to buzzer and/or electro shock was observed.

Cataleptic effects

In pilot studies, effects of 4 and 8 mg/kg body wt i.p. of KF were observed to induce cataleptic stances in some of the animals. So any cataleptic effect per sec of the extract was evaluated by the following method. Catalepsy was first qualitatively measured by the staging system and later quantified by the "ring test". Four distinct stages are discernable which are attained gradually.

Stage I - animals site quietly, without any attempt to move, unless pushed gently.

Stage II - animals does not move on being pushed.

Stage III -animal maintains an abnormal posture, in which its forepaws are gently placed on lower retort ring (7.5 cm high) for 30 seconds or more.

Stage IV-animals maintains for 30 seconds or more, an abnormal posture, where one of its forepaws is lifted and/or one of the hind paws remained suspended in the air.

Once the animal attained stage IV or the deepest attainable stage, it was subjected to the "ring test" of Pertwee (1972)[13], where the animals were placed gently on the upper ring (40 cm hitch) of the cataleptic stand" in a manner that the paws rested on the ring, their hand facing away from the vertical stand. The animal was closely observed for a total period of 5 minutes during which the animal remained perfectly immobile (the cessation of the stout and wiskering movements taken as index) with the exception of respiratory excursion and a typical sagging movement, was recorded to the nearest second with a stop watch. Another stop watch was used to mark the passage of total period of observation (5 minutes). The period of complete immobility gave the "immobility index" and was converted into "percentage immobility".

RESULTS

1. Observation KF1, KF2 and hexobarbitone induced sleeping time in mice

Hexobarbitone or ultra short-acting barbiturate is a well known hypnotic suitable for experimentation because of its short duration of sedation. It was observed on intraperitoneal administration of KF1 and KF2 to study the effects on gross behaviour in albino mice, that the animals became very quiet and subdued for a considerable period of time. This observation led us to study the effects of the crude extract on hexobarbitone induced sleeping time in mice. The results are tabulated in Table 2 & 3 and as depicted in Figures 3 & 4.

Table 2: Effects of KF1 and KF2 on hexobarbitone (100mg/kg b.w., i.p), (Hexo 100) induced sleeping time in mice

(Each observation is an average of 5 animals)

Group	Sleeping time Mean ± S.E.	p value
Hexo 100 mg	30.0± 0.62	-
Hexo 100 mg + KF1 - 4mg	42.0 ± 0.68	(0.001)
Hexo 100 mg + KF1 - 8mg	46.0 ± 0.80	(0.001)
Hexo100 mg + KF1 - 16mg	52.0 ± 0.84	(0.001)
Hexo 100 mg + KF2 - 4mg	30.4 ± 0.60	N.S*
Hexo 100 mg + KF2 - 8mg	31.6 ± 0.48	N.S*
Hexo 100 mg + KF2 - 16mg	32.0 ± 0.66	N.S*

Note - N.S* denotes in comparison to control group, Student t-test.

Figures 3

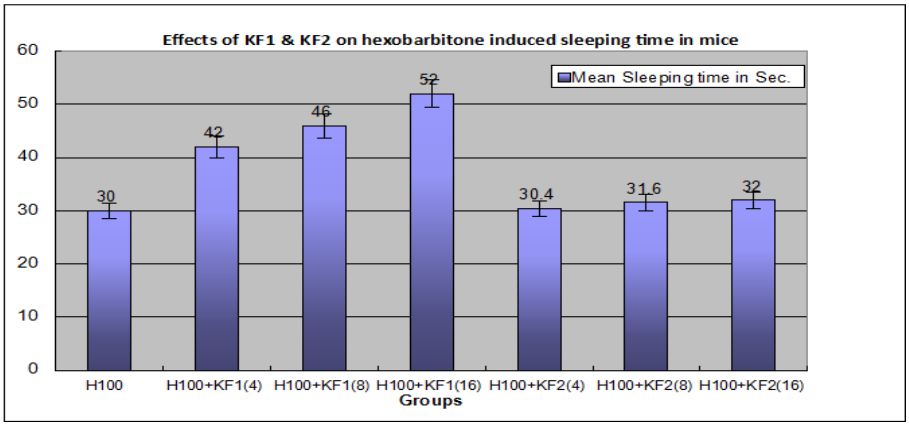
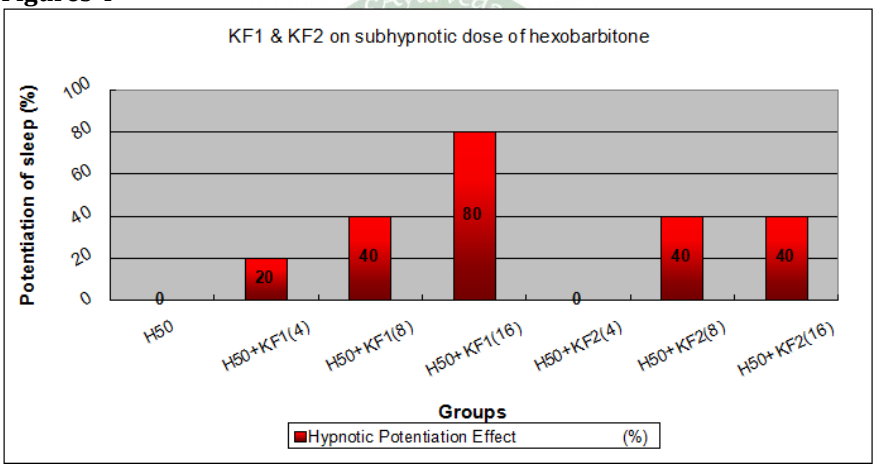


Table 3: Observations on the effects of KF1 and KF2 on sub hypnotic (50 mg/kg. b.w., i.p) dose of hexobarbitone (Hexo 50)

Group	Hypnotic Potentiation Effect (%)
Hexo 50 mg	00
Hexo 50 mg + KF1 - 4mg	20
Hexo 50 mg + KF1 - 8mg	40
Hexo 50 mg + KF1 - 16mg	80
Hexo 50 mg + KF2 - 4mg	00
Hexo 50 mg + KF2 - 8mg	40
Hexo 50 mg + KF2 - 16mg	40

(Each observation is an average of 5 animals)

Figures 4



From the above tables it was observed, that KF1 in all doses i.e. 4, 8 and 16 mg/kg. b.w. i.p potentiated the hexobarbitone induced sleeping time in mice. However, KF2 failed to produce similar increase in sleeping time.

2. Observations On The Analgesic Effect of KF1 AND KF2

Potentiation of sleeping time in mice by KF1 led us to undertake the present study, to observe whether KF1 and KF2 posses any analgesic effect or not. The drug extract was compared with sub-analgesic dose of 2 mg/kg. i.p) as standard anti-nonceptive agent. The results are tabulated in Table - 4.

Table 4: The effect of KF1 and KF2 on sub analgesic dose of M_S

Groups	Increase in latent period of tail flick response (in sec.) - Mean ± S.E.	p value
Morphine (M_S)	1.46 ± 0.32	-
M_S + KF1 (4 mg)	1.52 ± 0.34	N.S*
M_S + KF1 (8 mg)	1.48 ± 0.28	N.S*
M_S + KF1 (16 mg)	1.60 ± 0.34	N.S*
M_S + KF2 (4 mg)	1.44 ± 0.36	N.S*
M_S + KF2 (8 mg)	1.46 ± 0.30	N.S*
M_S + KF2 (16mg)	1.54 ± 0.34	N.S*

- N.S* denotes in comparison to control group, Student t-test.

(Each observation is an average of 5 animals)

KF1 and KF2 in all doses failed to produce no analgesic effect and also did not prolong the sub-analgesic dose of morphine.

3. Observations On The Spontaneous Motility In Mice

To study the effect of KF1 and KF2 on the spontaneous motility in mice, the animals were kept in the "Jingling cage" and their normal movements were recorded. Then groups of animals containing 5 mice each, were injected intraperitoneally with KF1 and KF2 in doses of 2, 4, 8 and 16 mg/kg. b.w. respectively and the remaining group was treated with propylene glycol and served as control. In a dose of 2mg/kg. b.w. both KF1 and KF2 failed to produce any observable effect on the spontaneous motility of mice. Although doses 4 mg and 8 mg/kg.b.w. of KF1 markedly diminished the spontaneous movement and the exploratory movement of the mice was totally abolished. the mice in this group preferred to remain seated in the corners of the cages and this effect lasted almost 3 hours. In similar doses, KF2 however failed to produce any discornable effect on the spontaneous motility of the mice.

When 16mg/kg. b.w. of KF1 was administered there was complete cessation of movements of the mice and the effect lasted for almost 4 hours. The drug solution, KF2 in doses of 16 mg/kg. b.w., reduced the motility in mice, but the effect was rather short-lasting, lasting only 1 hour.

4. Observations On The Rota Rod Test

It was observed that KF1 in doses of 2 mg/kg. b.w. did not significantly affect the motor activity of the mice. However, in higher dose i.e. 8 mg/kg. and 16 mg/kg. body weight, all the mice fell down more than twice, when the period of observation was kept fixed at 3 minutes. This observation was found to be statistically significant. This suggested that KF1 in 8 mg/kg. considerably affected the motor activity in mice, KF2, however, in doses of 2.8 mg did not significantly reduce the motor activity in mice. But in the dose of 16mg/kg. body wt. KF2, 3 out of 5 mice fell for twice in 3 minutes observation. The results are tabulated in Table - 5.

Table 5: Observations on the effect of KF1 and KF2 on motor activity of mice as determined by Rota rod test Each group contain 5 albino rats)

Groups (Dosage in mg/kg b.w.)	No. of falls by rats in each group during 3 minutes stay	Mean ± S.E.	p value
N. Saline (1 c.c.)	02	04 ± 0.20	-
KF1 2	03	06 ± 0.34	N.S*
8	12	2.4 ± 0.36	(0.01)
16	16	3.2 ± 0.42	(0.001)
KF2 2	02	04 ± 0.24	N.S*
8	03	2.4 ± 0.36	N.S*
16	06	1.2 ± 0.38	N.S*

Note -N.S* denotes in comparison to control group, Student t-test.

5. Evaluation Of The Sedative Ataxic Score

The mean of the scores for each group of animals was calculated and tabulated in Table - 6 and reveals that the sedative ataxic score for control group of animals was zero. While, animals treated with drug KF1 in the doses of 4 mg/kg. and 8 mg/kg. body weight respectively, the score was 1.4 and 2.6 respectively. The drug solution KF2, which was administered in similar doses of 4.8 mg/kg. body wt. produced a score of 0.2 and 0.6 only. While pentobarbitone sodium in the doses of 4 and 8 mg./kg b.w. produced a score of 2.6 and 4.4 respectively.

Table 6: Observation on the Sedative ataxic score in mice (Each group consists of five animals)

Groups	Drug	Dose (mg/kg b.w. i.p)	Sedative ataxic score Mean ± S.E	p value
A	NS	1 ml	0	-
B	KF1	4	1.4 ± 0.24	<0.001
C	KF1	8	2.6 ± 0.42	<0.001
D	KF2	4	0.2 ± 0.12	N.S*
E	KF2	8	0.6 ± 0.20	<0.05
F	Pentobarbitone	4	2.6 ± 0.48	<0.001
G	Pentobarbitone	8	4.4 ± 0.60	<0.001

Note - N.S* denotes in comparison to control group by Student t-test.

From the above table it can be observed that the sedative ataxic score was highest with pentobarbitone, moderate with drug solution KF1 and least with KF2.

6. Observation On Behavioural Activities

Gross Behavioural Activities

It was observed that KF1 in doses of 2.4/mg/kg. b.w. produced considerable CNS depression within 30 minutes of administration. The mice stopped all exploratory movements wiskering was diminished and totally stopped grooming

itself. The mice also developed ataxic movement and its gait was flattened and these effects lasted the whole period of observation. At similar doses KF2 however, produced only slight diminution of exploratory movements in mice, but it reduced wiskering and grooming habits of the animal and produce minimal ataxia. These effects lasted for more than 1 hour only. In the dose of 8 mg/kg. b.w. of KF1, the animals became deeply sedated when observed at 30 minutes interval after administration. The righting reflex was preserved but the mice attained some abnormal postures, while these effects lasted over 3hours. While KF2 in a similar dose of 8 mg/kg. b.w. dose produced diminution of exploratory movement and the wiskering and grooming habits of the animals were stopped and also its gait was flattened. The animals however, did not lose the righting reflex or develop abnormal postures. In the control group of mice, which was treated with normal saline 1 ml. intraperitoneally, did not alter the normal gross behavioural activities of the mice.

Conditioned Avoidance Response In Rats

The drug KF1 had previously shown a quietening and hypnotic effect in rats and mice. Therefore, it was felt necessary to further study this hypnotic effect and hence the evaluation of the conditioned avoidance response using the "jumping box" technique was undertaken. The drug KF1 and KF2 were compared with known sedatives/tranquillizers e.g. chlorpromazine and pentobarbitone, Piala et al., (1959)[12] first used the "jumping box" technique and this method was followed here. A peculiar phenomenon was noticed whereby excessively trained rats who were showing "secondary response" i.e. jumping to the other side of the box, even on just putting the rat in the jumping box, before even the buzzer was sounded. This effect is blocked by anti-anxiety drugs like benzodiazepines. KF1 in higher doses i.e. 16 mg/kg. body wt. and above blocked this secondary response in rats. Both the drug solution KF1 and KF2 failed to produce any effect on the conditioned avoidance response in rats.

Catalepsy Test

To quantify the catalepsy, as observed when K.fruticosa was administered in dose of 4 and 8 mg/kg. body wt. during pilot studies, the "ring test" of Pertwee (1972)[13] was performed, and the results are tabulated in Table - 7.

Cataleptic (5.0 mg/kg. body wt. i.p) and subcataleptic (0.3 mg/kg. body wt.) doses of haloperidol were choosen. Haloperidol in 5 mg/kg. body wt dose induced moderate catalepsy in all treated animals, most of the attained at least stage III, KF1 in doses of 2 and 4 mg/kg. b.w. significantly potentiated, but in doses of 8 and 16 mg. b.w. effected maximum potentiation as all animals attaining Stage IV, while KF2 did not potentiate cataleptic dose of haloperidol.

Further 0.3 mg/kg. body wt. of haloperiodol i.p. does not induce significant catalepsy in most mice, with only few animals attaining Stage II. KF1 was given in dose of 2, 4, 8 and 16 (mg/kg. body wt. i.p.) along with sub cataleptic dose of haloperidol. The animals attaining mostly stage III, where 2 out of 5 animals in the group with 16 mg dose of KF1 attained stage IV, but KF2 did not potentiate sub cataleptic dose of haloperidol at all. The observations are tabulated in Table - 7

Table -7: **Effect of KF1 and KF2 on cataleptic and sub-cataleptic doses of haloperidol**

(Each observation is an average of 5 rats)

Drug with dose (in mg/kg b.w.)	Cataleptic score	P Value
Halo	63.4 ± 2.44	-
Halo + KF1 (2)	82.4 ± 1.26	<0.001*
Halo + KF1 (4)	89.4 ± 2.86	<0.001*
Halo + KF1 (8)	89.8 ± 2.28	<0.001*
Halo + KF1 (16)	94.6 ± 1.82	<0.001*
Halo + KF2 (2)	63.8 ± 2.42	N.S*
Halo + KF2 (4)	64.2 ± 2.44	N.S*
Halo + KF2 (8)	65.2 ± 2.38	N.S*
Halo + KF2 (16)	65.4 ± 2.42	N.S*
$Halo_S$	25.3 ± 2.86	-
$Halo_S$ + KF1 (2)	42.6 ± 3.24	<0.001+
$Halo_S$ + KF1 (4)	48.4 ± 4.26	<0.001+
$Halo_S$ + KF1 (8)	74.8 ± 4.66	<0.001+
$Halo_S$ + KF1 (16)	76.4 ± 4.68	<0.001+
$Halo_S$ + KF2 (2)	24.6 ± 2.32	N.S*
$Halo_S$ + KF2 (4)	26.6 ± 3.42	N.S*
$Halo_S$ + KF2 (8)	44.2 ± 3.56	N.S*
$Halo_S$ + KF2 (16)	62.2 ± 3.58	N.S*

(Halo=Cataleptic dose of haloperidol - 5 mg/kg. b.w. i.p.)

($Halo_S$ = Sub-cataleptic dose of haloperidol - 0.3 mg/kg. b.w. i.p.)

'*' Statistical significance in comparison to 'Halo' group

'+' Statistical significance in comparison to '$Halo_S$' group

N.S* = Not significant, Student t-test.

DISCUSSION

The plant being investigated, i.e. *Kopsia fruticosa* (KF), in our studies have been reported to yield four different alkaloids, isolated from different parts of the plant. But all of them have indole moiety as basic nucleus. In the field of studies of indigenous plants, several potent pharmacological actions have been attributed to the presence of the indole alkaloids in the extracts of the plants. Though reports about pharmacological actions of KF are scanty, till now they have been reported to induce cholinergic actions[14]. Paucity in the previous literature regarding KF alkaloids prompted us to investigate the effects of the extracts of this plant on cardiovascular system, respiratory system, central nervous system as also effects on skeletal and smooth muscle preparations, as other members of Apocynaceae family have been reported to induce varieties of pharmacological effects like depression of the CNS and CVS, vasodilatory, local anaesthetic and others[1]. As this plant is not a native of even Indian subcontinent, being found mostly in Malaysian peninsula. Enormous and continuous supply of the plant is not there, so we concentrated on the effects of the total extract of leaves of KF.

Two different vehicles were used for preparing the extracts of the leaves of KF, namely, propylene glycolic solution of the extract designated as KF1 and aqueous solution of the extract designated as KF2, were made. Evaluating the effect of KF1 and KF2 on spontaneous locomotor activity in mice it was seen that both the extracts had a dampening effect on the locomotor movements of mice as seen by testing them in the "Jingling cage". Effect wise KF2 was quite less effective than KF1. Both the spontaneous movements as well as exploratory behaviour were diminished. Mice treated with the extract were mostly cornered huddling together at the corners of the animal cage, with higher doses, this effect persisted for at least 4 hours after administration of the extract, showing a central nervous system depressant action. In rotating rod test at lower doses, there was not much effect but in higher doses, that is 8 mg/kg. body wt. dose of KF1 and to fall of the tested rats, which fell down more than twice during the period of observation. KF2 was not much effective in this parameter. On evaluation of the sedative ataxic scores of KF1 and KF2 on mice and comparing them with the same effect of pentobarbitone, it was seen that KF1 had a moderate sedative ataxic effect, though not at par with pentobarbitone, KF2, in all doses was least effective.

In our study with rats about the effect of KF1 and KF2 on the conditioned avoidance response induced in the animals by the 'jumping box' method. Though the extract, showed calming effect but they did not modify the response in trained rats showing conditioned avoidance response. One peculiarity, a phenomenon was seen whereby excessively trained rats who were showing "secondary response" i.e. jumping to the other side of the box, even on just putting the rat in the jumping box, before even the buzzer was sounded. This is a response which is blocked by anti-anxiety drugs like benzodiazepines, KF1 in higher doses show a similar effect of blocking the 'secondary response' without affecting the conditional avoidance response.

Observations of the effects of KF1 and KF2 on hexobarbitone induced sleeping time in mice, it was seen KF1 in all doses potentiated the hypnotic dose of hexobarbitone as well as the sub-hypnotic dose of hexabarbitone, also the subhypnotic dose of hexobarbitone could not induce sleep in any of the animals while there was dose dependent increase in the percentage of induction of sleep with KF1. KF2 was totally ineffective in modifying the response of both hypnotic as well as sub-hypnotic dose of hexobarbitone.

Testing the drugs on the analgesiometer for their anti-nociceptive effect if any, both the solutions of the extracts were found to be devoid of any anti-nociceptive effect per se. Moreover, they could not potentiate sub-hypnotic dose of morphine.

Observing the effects of KF1 and KF2 on the gross behavioural activities of mice, it was found that with KF1, the mice showed considerable central nervous system depression. The animals stopped all exploratory movements, wiskering was diminished and they stopped grooming themselves completely. The mice also developed ataxic movements and even attained abnormal postures. At similar doses, however, KF2 produced marginal decrease in motility of the mice with reduced wiskering and grooming, but no abnormal posture or ataxia was observed.

KF1 per se induced a moderate degree of catalepsy in the rats and also potentiated cataleptic and subcataleptic doses of haloperidol. Whereas KF2 could not potentiate haloperidol in its cataleptogenic action. It is too early, to conclude about the exact mode of action of the extract of KF, but seeing the ineffectiveness, of the two vehicles of the extract i.e. propylene glycol and distilled water in affecting in any way the different parameters as discussed above, on which the animals were tested, it can be postulated that KF1 has an central nervous system depressant, sedative, ataxic, cataleptic but not affecting the conditioned avoidance response (like minor tranquillisers) actions. KF2 was almost devoid of any effect on these CNS paradigms. Both KF1 and KF2 however, showed depressant effect as per haemodynamic and cardiac effects are concerned. It is very surprising, that the aqueous extract shows minimal CNS effects as compared to the glycolic extract, KF1. The reason cannot be explained at this stage and requires a further exploration and insight into their pharmacological properties.

CONCLUSION

Both the solutions viz. propylene glycolic (KF1) aqueous (KF2) and extracts obtained from the leaves of *Kopsia fruticosa* have been studied in the present study. KF2 had dampening effect on the locomotor system. The spontaneous movements were diminished and there was a distinct calming effect. KF1 showed considerable CNS depressant effect which persisted for almost 4 hours. KF2 or the aqueous solution of the crude extract produced minimal effect in these parameters. KF1 potentiated the hexobarbitone induced sleeping time in mice and even

potentiated the subhypnotic dose of hexobarbitone. But KF2 had no sedative effect and did not potentiate the hexobarbitone induced sleeping time in mice. Both the extracts, KF1 and KF2 had no analgesic effect. They also did not alter the conditional avoidance response in mice. However, KF1 produced a moderate degree of catalepsy and potentiated even the sub cataleptic dose of haloperidol. Hence, the present study with *Kopsia fruticosa* shows some interesting pharmacological effects with KF1, the propylene glycolic solution of the crude extract that possess CNS depressant, sedative, ataxic, cataleptogenic actions. While the aqueous solution of the crude extract had very little action on CNS paradigms.

AUTHORS' CONTRIBUTION

The present work was conceived and conducted by DB and SM, statistical analysis was done by SM, RSD has prepared the manuscript that was subsequently approved by all the co-authors, and TG performed the referencing task.

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