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Central nervous system activity of *Illicium verum* fruit extracts

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ABSTRACT

Objective: To research the acute toxicity of *Illicium verum* (*I. verum*) fruit extracts and its action on central nervous system. **Methods:** The TLC and HPTLC techniques were used as fingerprints to determine the chemical components present in *I. verum*. Male albino rats and mice were utilized for study. The powdered material was successively extracted with *n*-hexane, ethyl acetate and methanol using a Soxhlet extractor. Acute toxicity studies were performed as per OECD guidelines. The CNS activity was evaluated on parameters of general behavior, sleeping pattern, locomotor activity, anxiety and myocoordination activity. The animals were trained for seven days prior to experiments and the divided into five groups with six animals in each. The drug was administered by intraperitoneal route according to body weight. The dosing was done as prescribed in each protocol. **Results:** Toxicity studies reported 2 000 mg/kg as toxicological dose and 1/10 of the same dose was taken as therapeutic dose Intraperitoneal injection of all extracts at dose of 200 mg prolonged phenobarbitone induced sleeping time, produced alteration in general behavior pattern, reduced locomotor activity and produced anxiolytic effects but the extracts do not significantly alter muscles coordination activity. The three extracts of *I. verum* at the dose of 200 mg, methanol extract was found to produce more prominent effects, then hexane and ethylacetate extracts. **Conclusions:** The observation suggested that the extracts of *I. verum* possess potent CNS depressant action and anxiolytic effect without interfering with motor coordination.

1. Introduction

Illicium verum (*I. verum*) belonging to the Magnoliaceae family, commonly known as Chinese star anise is one of the flavors used in china 5 spices, cultivated in mountainous region especially in Lanson province, Cochin, China (Southern china) and Vietnam[1]. The *I. verum* fruits are capsule like aggregate with star shaped five to ten pointed boat shaped section about on eight averages. Each arm is a seed pod. The fruits have tough skin and rust colored outer portion and seeds with high oil content[2,3]. The oil is used in rheumatism[4]. The volatile oil and acetone extract of *I. verum* shows significant antioxidant and antimicrobial effects[5].

The fruit contain higher bitter principle, tannins and essential oil (9%-10%), consisting of anethole (85%-90%), α-pinene, limone, β-phellandrene, α-terpineol, farnesol and safrol[6]. The chemical analysis of *I. verum* essential oil 16 compounds were identified confirms presence of linalool and estragole, essential oil also possess antifungal activity[7]. They are 14 hydrocarbons components and 22 oxygenated hydrocarbon derivatives and small amount of nitrogenous compounds ρ-allylanisole, ρ-cumicaldehyde, ρ-allylpen, anisylacetone, anisaldehyde, linoleic acid (1-4 methoxyphenyl)-prop-2-one), foeniculin and palmitic acid[8]. The new phenylpropanoid glycosides, known as SecoCycloartane; 3, 4 seco (242) cycloartane 4(28), 24 (diene) 3, 26-dioicacid. 26, methyl ester of nigranoic acid from the dichloromethane

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extract from leaves of *I. verum* was identified[9,10].

The three new neurotropic sesqiterpenoids, veranistains A, B, C were isolated and pharmacological activities of veranistains and anisatin was evaluated for locomotor and analgesic activities[11]. Although preliminary pharmacological studies with *I. verum* have been undertaken, there are no data about the pharmacological effects of this species on behavior and central nervous system (CNS). Therefore, the present work was undertaken to evaluate the neuropharmacological effects of polar and non polar extract of *I. verum* on different animal models.

2. Materials and methods

2.1. Animals

Healthy male albino rats weighing 80-120 g and mice 25-30 g was utilized for the acute toxicity test and CNS activity. The animals were obtained from animal house of V.N.S Institute of Pharmacy, Bhopal. Ethical clearance for handling of animals and procedures used in study was obtained from the institutional animal ethical committee prior to the beginning of the study (Reg no: 778/03/c/CPCSEA). All animals were stored in standard cages and maintained at (27±2) ℃ under 12 h dark/light cycle. The animals were fed with standard rat feed and water was given after specific interval, prior to experiment animals were kept for 12 h fasting.

2.2. Plant material and preparation of extracts

The dried fruits of *I. verum* were collected from local market of Bhopal, (M.P) and identified and authenticated from Safia college of science as Voucher Specimen No.136/Bot/Saf/BPL by Dr. Zia UI Hassan, Safia college of science, Peer gate, Bhopal (M.P), India. The dried fruits were coarsely powdered. The powdered material (190 g) was successively extracted with *n*-hexane, ethylacetate and methanol using a Soxhlet extractor, the extracts were concentrated in a rotatory evaporator under reduced pressure to afford dry yield of 9.38 g (6.26%) of *n*-hexane extract (HX), 9.53 g (5.01%) of ethylacetate extract (ET), 28.84 g (15.17%) of methanol extract (MH) were dissolved completely in DMSO.

2.3. Drugs and chemicals

Diazepam (Valium 2 mg tablet, Roche products pvt ltd, India) was used as the reference drug in all the animal models studied. It was dissolved in DMSO for *ip.* injection. Other solvents used were of analytical grade.

2.4. Phytochemical investigation

The *n*-hexane, ethyl acetate, methanol are subjected to phytochemical analysis using conventional protocol[12].

2.5. Chromatographic studies of extracts

2.5.1. Thin-layer chromatography

Out of the various solvent systems tried, chloroform: methanol: Water (4:3:2) gave the best resolution (number of spots *I. verum* = 4), the detecting reagent was anisaldehyde in sulphuric acid followed by heating at 110 ℃ for 5 min.

2.5.2. Selection of HPTLC plates

Precoated and preactivated TLC plates of silica gel 60 F254+366E. (MERCK KGaA), with the support of aluminum sheets having thickness of 0.1 mm and size 10 cm×10 cm, were cut smaller according to required dimensions ,the prewashing was done with methanol, and drying at 120 ℃ for 20 min[13].

2.5.3. Sample preparation

Hexane, ethylacetate and methanol extract of *I. verum* fruits (20 mg) were dissolved in 1 mL of solvent used for their extraction and 10 μL of sample were applied as band length 5-8 mm from the lower edge of the plate using 100 μL syringe on CAMAG COMPANY, SWITZERLAND automatic sample applicator.

2.5.4. Application of sample

The extract samples was applied in the form of a band using CAMAG LINOMAT IV, an automatic sample application device, maintaining a band width 6.0 mm, space 8.0 mm, 150 μL/s. The quantity of sample applied was 10 μL. The following mobile phase was selected experimentally, Toluene: Chloroform: Acetone (40:25:35). The plates were developed by placing in a presaturated tank (12 cm height) with the mobile phase for 2 h. The plates were dried by evaporating the solvent either at room temperature or by spraying hot air by air dryer.

2.6. Acute toxicity

Acute toxicity studies were carried out using acute toxic class method as per OECD guidelines 423[14]. Acute toxicity for various plant extracts was carried out using groups of three Swiss albino mice by administering varying doses of (0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00 g/kg) in DMSO *po.* while the control group received only the vehicle. The groups were observed mortality and behavioral changes during 48 h.

2.7. Pharmacological evaluation

2.7.1. General behavioral tests

Swiss albino mice were divided into five groups (6 in each group). The first three groups were injected intraperitoneally with *n*-hexane, ethyl acetate and methanol extracts with 200 mg/kg dose each and fourth received DMSO as vehicle, whereas fifth group received Diazepam (2 mg/kg *ip.*) which served as a standard drug. The activities were recorded at

30-min intervals in the first hour and at hourly intervals for the next 4 h for the following parameters.

Spontaneous activity, awareness and alertness: These were evaluated by placing a mouse in a bell jar. It usually shows a moderate degree of inquisitive behavior.

Sound responses: Mice normally utter no sound, so that vocalization may point to a noxious stimulus.

Touch responses: It was noted when the animal was touched with a forceps (or) pencil at various parts (*ie.* on the side of the neck, on the abdomen and on the groin).

Pain response: This response was graded when a small artery clamp was attached to the base of tail[15,16].

2.7.2. Locomotor activity

The locomotor activity (horizontal activity) was measured using an actophotometer. The movement of the animal cuts off a beam of light falling on the photocell and a count was recorded and displayed digitally. Each rat was placed individually in the actophotometer for 10 min and basal activity score was obtained. Subsequently, the animals were divided into 5 groups each group consisting of 6 animals, and diazepam 2 mg/kg, *ip.* (positive control group) were administered and after 60 min the rats were placed again in the actophotometer for recording the activity score as described earlier[17].

2.7.3. Effect on motor coordination

The animals were trained to maintain balance for 2 min on the rod rotating at the speed of 20 rpm. Only those rats, which could balance themselves, were selected for the study. Each rat was placed individually on the rota rod and the total number of falls within 2 min was noted, which was considered as the basal reading. Subsequently, the animals were divided into five groups, each group consisting of six animals. One hour following the administration of DMSO as vehicle, hexane (200 mg/kg), ethyl acetate (200 mg/kg), methanol (200 mg/kg) and diazepam (2 mg/kg, *ip.*) the rats were again placed on the rota rod and the number of falls per 2 min were recorded[18].

2.7.4. Effect on phenobarbitone sodium sleeping time

Swiss albino mice were divided into four groups (6 in each). The extracts were injected intraperitoneally to each group. Thirty minutes after the administration, each animal was injected with phenobarbitone sodium (40 mg/kg *ip.*). The sleeping time was noted by recording the time interval between the loss and return of righting reflex[19].

2.7.5. Elevated plus maze test (EPM)

The EPM for mice consisted of two perpendicular open arms (30 cm×5 cm) and two closed arms (30 cm×5 cm×2×5 cm) also in a perpendicular position. The open and closed arms were connected by a central platform (5 cm×5 cm). The platform and the lateral walls of the closed arms were made of transparent acrylic. The floor was made of black acrylic. The maze was 45 cm above the floor. Thirty minutes or 1 h after intraperitoneal injection of extracts respectively, the animal was placed at the centre of the plus maze with its nose in the direction of one of the closed arms, and observed for 5 min, according to the following parameters: number of entries in the open and closed arms, and time of permanence in each of them. The time of permanence measures the time spent by the animal in the open and closed arms. An increase in the total time spent into open-side arms indicated anti-anxiety response. Diazepam was used as positive control[20,21].

2.8. Statistical analysis

All the results were expressed as mean±SEM, and treated groups were compared with controls and difference was estimated by means of ANOVA followed by Dunnets test for multiple comparisons. P <0.05 was coonsidered as statistical significance.

3. Results

3.1. Phytochemical investigation

Phytochemical studies of the *I. verum* fruits exhibited various phytoconstituents as shown in Table 1.

3.2. Chromatographic studies

The HPTLC profile of methanolic extract of *I. verum* revealed the presence of 14 spots with R_f 0.03 (6.97 μg), 0.08 (112.93 μg), 0.13 (18.77 μg), 0.19 (15.74 μg), 0.27 (4.72 μg), 0.34(3.83 μg), 0.40 (12.80 μg), 0.51 (6.34 μg), 0.58 (12.48 μg), 0.65 (9.28 μg), 0.76 (7.87 μg), 0.79 (8.99 μg), 0.86 (14.16 μg), 0.99(53.44 μg) and component 2 with the maximum concentration of 112.93 μg at R_f 0.08 (Figure 1).

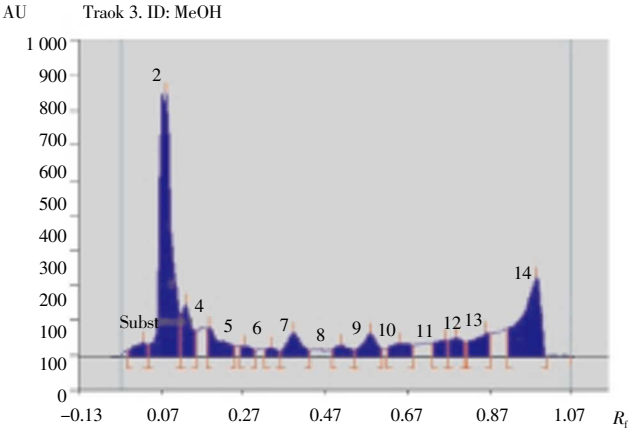

Figure 1. HPTLC chromatogram of methanol extract of *I. verum* at 254 nm.

Table 1

Qualitative analysis of various extracts of *I. verum* hook fruits.

Phyto-constituent	*n*-hexane extract	Ethyl-acetate extract	Methanol extract
Alkaloids	−	+	++
	−	−	++
	−	+	−
	−	+	+
Glycosides	−	−	+
	+	−	++
	+	−	+
	−	+	++
Protein and amino acids	−	−	−
	−	−	−
Sterols	+	++	+
	+	++	++
Carbohydrate	−	+	+
	−	+	+
Phenols	+	+	+
Flavanoids	−	+	+
	−	−	−
Tannins	−	−	++
	−	−	++
	−	−	−
Saponin	−	−	++
Triterpenoids	++	+	+

3.3. Acute toxicity

Acute toxicity for various plant extracts was carried out using groups of three Swiss albino mice by administering varying doses of (500, 750, 1 000, 1 250, 1 500, 1 750, 2 000 mg/kg) in DMSO *po*., while the control group received only the vehicle. The groups were observed mortality and behavioral changes during 48 h. Toxicity studies reported 2 000 mg/kg as toxicological dose b.w. 1/10 th of the same dose for all extract were taken as therapeutic dose *ie.* 200 mg/kg.

3.4. Effects of general behavioural profile

It was found that *I. verum* extracts affected the spontaneous activity, sound, touch, pain responses at the dose of 200 mg/kg and produced moderate/slight depression in animal models (Table 2).

Table 2

Effect of various extracts of *I. verum* on general behaviour (*n*=6).

Behaviour type	Hexane extract	EtoAc extract	MeoH extract
Spontaneous activity	+	++	+++
Alertness	++	+++	++
Awareness	++	++	+++
Sound response	++	+	+++
Touch response	+	++	++
Pain response	+	+++	+++

−: no effect; +: slight depression; ++: moderate depression; +++: strong depression; ++++: very strong depression.

3.5. Effect of I. verum fruits extract on spontaneous locomotor activity

The effect of plant extracts on locomotor activity was measured after 60 min when placed again in the actophotometer for recording the activity score as described earlier. Locomotor activity was decreased in animals injected with *I. verum* (200 mg/kg), compared with controls. The administration of diazepam at 2 mg/kg also significantly suppressed the locomotor activity (Table 3).

Table 3

Effect of various extracts of *I. verum* on locomotor activity (*n*=6).

Treatment (mg/kg)	Locomotor activity (scores) in 10 min		
	Before treatment	After treatment	Reduction in activity (%)
Control	109.60±5.45	73.16±5.80	33.24±2.26
MeoH. Ext.	110.66±17.81	44.10±4.30	60.14±3.25**
EtoAc. Ext.	119.83±10.65	52.40±2.60	56.27±3.33
Hexane. Ext.	99.83±10.65	47.00±1.30	52.91±3.11*
Diazepam (2.0)	100.33±10.65	30.12±3.41	70.21±10.65**

*P<0.05 and **P<0.01 *vs*. control (one-way ANOVA followed by Dunnett's test). Values represent mean±SEM.

3.6. Evaluation of anxiolytic activity using elevated plus maze apparatus

The elevated plus maze comprising of two open and two enclosed arms, produced a novel environment which helped in inducing anxiety in animals because of the open nature

Table 4
Antianxiety activity of various extracts of *I. verum* on elevated plus maze test in mice (n=6).

Group dose (mg/kg)	No. of entries (n)		Time spent (s)	
	Open arms	Closed arms	Open arms	Closed arms
Control	3.66±0.33	6.50±0.42	27.33±18.30	274.33±16.73
Hex Ext	6.33±0.49**	5.50±1.16	97.33±3.51*	202.67±3.52*
EtoAc.Ext	4.16±0.30	6.50±0.42	70.30±1.20	169.10±1.00
MeoH.Ext	8.50±0.42**	3.83±0.36	95.66±9.95*	203.33±9.93*
Diazepam	9.83±0.47**	3.50±0.79	131.77±31.72**	168.83±31.72**

*P<0.05, **P<0.01 *vs.* vehicle (one-way ANOVA followed by Dunnett's test). Values represent mean±SEM.

of the arms and elevation (70 cm) from the floor. When the animals were initially placed on the maze, they showed a preference for the enclosed (dark) arms and showed anxiety and fear like movements characterized by immobility, freezing and defecation on entering the open arms. The extracts 200 mg/kg, *ip.* extract produced significant increase in percent preference for open arm as first entry, in total number of entries in the open arm, as well as in the duration of stay in the open arm, when compared to the control group. Diazepam (2 mg/kg, *po.*) significantly increased (P<0.01) the percent preference for open arm, the number of entries as well as the duration of stay in the open arms, indicating anxiolytic activity (Table 4).

3.7. Potentiation of phenobarbitone sodium sleeping time

Treatment with hexane, ethyl acetate and methanol extracts in a 200 mg/kg, *ip.*, dosage lengthened the duration of the sodium pentobarbital-induced hypnosis in mice. The methanolic extracts significantly potentiated the phenobarbitone sodium induced sleeping time compared to respective control. The same effects were observed for the group treated with diazepam (2 mg/kg, *ip.*) (Table 5).

Table 5
Sedative effect of various extract of *I. verum* on (n=6).

Drug	Dose (mg/kg)	Duration of sleep (min)
Control	–	40.30±2.62
Phenobarbitol	40	47.20±5.23
MeoH.Ext +Phenobarbitol	200+40	75.40±7.62*
EtoAc.Ext + Phenobarbitol	200+40	65.60±4.12
Hex.Ext + Phenobarbitol	200+40	50.80±5.23

*P<0.05 *vs.* vehicle (one-way ANOVA followed by Dunnett's test).

3.8. Rotarod test

This test was performed to investigate whether the extracts were acting via the neuromuscular junction to mediate the observed effect rather than acting centrally. No alteration was observed on rotarod test after the treatment with all the extracts of *I. verum*, in contrast, diazepam (2 mg/kg, *ip.*) compared to controls shows myorelaxant properties as expected (Table 6).

Table 6
Effect of various extracts and diazepam on muscle-relaxant activity, studied using rota rod apparatus (n=6).

Treatment (200 mg/kg)	Number of falls in 2 min	
	Basal reading	After treatment
Vehicle	6.00±0.42	6.00±0.22
MeoH. Ext.	5.00±0.21	7.00±0.35
EtoAc. Ext.	4.00±0.53	8.00±0.56
Hex. Ext	4.00±0.35	6.00±0.44
Diazepam (2.0)	4.00±0.14	22.00±0.61**

*P<0.05, **P<0.01 *vs.* vehicle (one-way ANOVA followed by Dunnett's test). Values represent mean±SEM.

4. Discussion

In this work, the effect of polar and non polar extract of *I. verum* fruits were studied in several behavauriol animals model for the evaluation of central activity, and providing information about myorelaxant, anxiety and depressant activities.

The decrease in spontaneous motor activity is due to reduced excitability of CNS and this may be related to sedation resulting due to depression[22,23]. Our finding showed that all the extract decreased locomotor activity. The three extracts of *I. verum* exhibited varying degree of CNS depressant activity in the order of magnitude-MeoH>Hex>EtoAc. Mechanisms that possibly underlie this activity include activation of the inhibitory GABA ergic system[24].

All extracts at dose of 200 mg did not alter motor co-ordination in the Rota rod test, differently from diazepam (2 mg/kg) suggesting that the action of this plant may not exerted through peripheral neuromuscular blockage. Neuroleptics have inconsistent effect on sleep pattern, but tend to normalize sleep disturbance pattern[25]. The methanol extract (200 mg *ip.*) significantly potentiated the phenobarbitone sodium induced sleeping time possibily through a CNS depressant action. Decrease in spontaneous motor activity & potentiation of phenobarbital induced sleep strongly suggest central depressant activity.

Anxiolytic compounds reduce the natural aversion to

open arm and promote the exploration. In our investigation, the extracts produce changes in the exploratory activity in elevated plus maze model, but the stronger effect was observed in methanol than the hexane and ethyl acetate extracts. These results suggest that both polar and non-polar components are participating in the activity of the *I. verum* but polar substances apparently play a main role in the anxiolytic effects of this plant. Diazepam is a very well known anxiolytic benzodiazepine which produces important sedative effects.

The qualitative phytochemical studies revealed the presence of triterpenoids, steroids, flavanoids, phenols, saponin and alkaloids in plants extracts. The desired medicinal properties of the plant may be attributed to the synergistic or individual effects of these phytoconstituents which are well known for their neuropharmacological properties. The pharmacological studies have demonstrated that chemical constituents including flavanoids and essential oil displays nervous system depressant activity[26]. Anethole present in *I. verum* is traditionally reported to possess anxiolytic and sedative effects[27]. It has been suggested that volatile oils, either inhaled or applied to the skin, act by means of their lipophilic fraction reacting with the lipid parts of the cell membranes, and as a result, modify the activity of the calcium ion channels. They can interact with the cell membranes by means of their physiochemical properties and molecular shapes, and can influence their enzymes, carriers, ion channels and receptors. These include brain stimulation, anxiety-relieving sedation and antidepressant activities, as well as increasing the cerebral blood flow. The fragrance compounds are absorbed by inhalation and are able to cross the blood-brain barrier and interact with receptors in the central nervous system[28], whereas Flavanoids such as quercetin and kaempherol are also active constituents responsible for CNS depressant as anxiolytic[29,30]. Nevertheless, others components of flavonoid nature might play an important role in the pharmacological activity of this genus. So, in our studies TLC and HPTLC techniques were applied to determine the chemical composition of methanol samples and confirms the presence of 14 compounds which may be responsible for the activity. Glycosilation can strongly influence transport of flavanoids through haemato-encephalitic barrier, modifying the entrance into barrier tissue and its neuropharmacological properties[31,32]. It has also been described that they can be transported actively into brain using glucose transport system[33].

We showed that the extracts from fruits of *I. verum* has depressant and anxiolytic effect on CNS without interfering with motor coordination. whereas the extract has no toxicity upto 2 000 mg dose, therefore it is necessary to determine the evidence indicating depressant activity as well possible action mechanism.

Conflict of interest statement

We declare that we have no conflict of interest.

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