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HYPNOTIC EFFECT OF ESSENTIAL OIL AND METHANOLIC EXTRACT OF FRUITS OF *ZANTHOXYLUM BUDRUNGA* W.

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ABSTRACT: The study includes preliminary phytochemical investigations of n-hexane, ethyl acetate, methanol, and water extracts of fruits of *Zanthoxylum budrunga* W. The methanolic extract and essential oil were screened for hypnotic activities. Before performing hypnotic effect; other CNS effect like motor incoordination effect was also performed. Pretreatment with essential oil and methanolic extract (250 mg / kg, body weight) showed significant hypnotic activity ($p < 0.01$) comparable to the control.
Keywords: - Hypnosis, Mice, Thiopental, *Z. budrunga*.

INTRODUCTION AND EXPERIMENTAL

Zanthoxylum budrunga found very useful in many diseases, few of them already proved, bark, leave, fruit & seeds contain important medicinal- constituent's since study on different part reveal's its medicinal value, its plant from rutaceae family is small or moderate sized tree. The pericarp of unripe fruits is pleasantly aromatic & taste like the rind of a fresh orange. The fruit is hot & bitter. It's distributed in Konkan, Deccan, Mysore, Malabar, and Annamalais & Travarcore at low elevation, Orissa, Chittagong, Pegu. Also found in Assam & Meghalaya & in the eastern & western ghats in peninsular India[1]. The common names of plant at different places are; badrang, pepuli, mullilam[2]. The different parts of plant subjected for research work & whole plant found to have effective in many diseases. Since plant contains flavones i.e. 5-methoxy- 7-hydroxy flavol, essential oils, resins, alkaloids & lignans, & many others important phytoconstituents in its different parts, due to the presence of these components plant employed in many diseases, few of them already have been proved[1, 3, 4, 5]. The fruits are used as digestive appetizer, cure asthma and bronchitis, remove pain, useful in heart diseases, good for piles, useful in diseases of mouth, teeth & throat, also prescribed in dyspepsia & diarrhoea[1]. Essential oil from fruits showed significant local anaesthetic activity at 0.02% by infiltration & corneal application in guina pigs[6, 7]. Since fruits shows significant local anaesthetic activitity & traditionally it's also employed for sedative activity at higher dose[8], thus our aim of work was to evaluate fruit's methanolic extract & essential oil for hypnotic activity. Compounds with sedative activity may produces depressant effect like relaxation in muscular activity (motor incoordination) thus plant extract & oil were also evaluated for above mentioned activity before performing sedative-hypnotic activity.

MATERIALS AND METHODS

PLANT:

Collection of fruits of *Zanthoxylum budrunga* W. was done from the Mulsi village, Pune, India, in the month of Jan-Feb, 2008. Taxonomic identification of the plant has been done by Dr. S.S. Deokule, Professor Department of Botany, University of Pune (Boucher no. of authentication of plant145-7719 UNIP IN.). Fruits of plant *Zanthoxylum budrunga* W. were divided in two parts, one part were subjected to steam distillation after

pulverized to coarse powder directly, & another part dried in shade for few days & after complete drying this part was also pulverized to coarse powder in grinder & subjected to evaluation for different parameters & solvent extraction.

EXTRACTION:

DEFATTATION OF POWDERED FRUITS OF *ZANTHOXYLUM BUDRUNGA*:

50 gm of dried powdered was defatted with 300 ml of hexane (66-68^{o}c). The procedure was repeated for 10 times to get extraction of 500 gm powdered fruits of *Zanthoxylum budrunga* (each time 50 gm powdered material was extracted with hexane). After defattation, the marc was taken out from extractor and spread as a bed on a clean paper and dried till evaporation of hexane. The hexane extract was collected and kept for phytochemical analysis, separation and identification.

SUCCESSIVE EXTRACTIONS:

490 gm of dried marc obtained after defattation (from 500 gm powdered material) was packed in soxhlet extractor and extracted with ethyl acetate at 76^{o}C, again in 10 batches, each time extraction of 49 gm material performed by using 300 ml ethyl acetate. The extract was collected and the solvent was evaporated (to concentrate) from this, to obtained ethyl acetate extract. The marc was taken out from extractor and spreaded on a clean paper till the evaporation of solvent.

This dried marc (480.02 gm) was again packed in soxhlet extractor and extracted with methanol at 78°C in 10 batches (10 x 48.02 gm). The extract was collected and the solvent was evaporated from this methanol extract to get concentrated extract. The marc was taken out from extractor and spreaded on a clean paper till evaporation of methanol.

This dried marc (461.24) was again packed in soxhlet extractor and extracted with distilled water at 95^{0}c, in 10 dived batches, each batch contain 46.12 gm material with 300 ml water. It was extracted until a drop of solvent from the siphon tube when evaporated did not leave a residue. The extract was collected and the solvent was evaporated from this to obtained water extract. All the extracts (syrupy consistency) were concentrated on hot plate and stored for identification, isolation and pharmacological screening.

STEAM DISTILLATION:

Steam distillation was performed to get volatile oils from the fruits of *Zanthoxylum budrunga.* First the fruits 100 gm (out of 1000 gm, without drying) were crushed in mixer, and then placed in two distillation flask, each one contained 50 gm of fruits material. Then steam distillated, by passing steam from separate steam generator after distillation oil was collected. Procedure was repeated 10 times to get oil from 1000 gm fruits. Oil taken up in ether, filtered and then ether was removed by distillation; finally pure essential oil was obtained Every time 100 gm materials were distillated by using two distillation flasks, each one contained 50 gm drug material. Finally oil obtained from 1000 gm fruits are stored in well closed air tight bottle, covered with parafilm & stored in refrigerator.

ANIMALS:

Albino mice of either sex; weighing between 16-22 g body weight were obtained from institute animal house, used for hypnotic & other CNS depressant activity[9,10]. The Institutional animal ethics committee approved the employed protocol (Reg. no. 541/02/C/CPCSEA. (from animal committee to college) Ref. no. LMC/PHARM/OFFICE/2008-2009/914.Date: 16/02/09). They were fed with standard diet and maintained under standard laboratory condition (12 hour light/ 12 hour dark cycle; (25 ± 3°) and had free access to drinking water.

Mice were divided in to 4 groups of 6 each for hypnotic activity and the drugs were injected as per following protocol:

1. Methanolic extract (150 mg/kg dose, ip)
2. Methanolic extract (250 mg/kg dose, ip)
3. Essential oil (0.2 ml of 0.25% oil in saline, ip)
4. Saline as control (10 ml/kg, ip)

STATISTICAL ANALYSIS:

The data are expressed as mean±SE. comparison of sleeping time in all groups was made using ANOVA. Significant was measured finally between test & control group.

RESULTS AND DISCUSSION

MUSCLE RELAXANT ACTIVITY:

"Rota rod" apparatus was used to determine motor coordination of methanolic extract & essential oil. Rotarod a rotating-rod, grip of mice on rotating rod is due to the muscle grip strength, C.N.S. depressant drug decreases the grip strength & mice may fall from the rotating rod due to the effect of drug. Loss of grip strength measured as motor in coordination or muscle relaxant effect of drug[11, 12]. Animals were first treated with test & control drug through i.p. route of administration, after 30 min. of dosing animals were placed on rotating rod (13-15 rpm) for 1 min. Number of animals fall from rod with in this period were counted as the loss of grip strength. To measure motor in coordination (muscle relaxant activity) of drug % of animals that fall from rod was compared between test & control groups (Table 1).

MOTOR IN COORDINATION EFFECT OF METHANOLIC EXTRACT:

% of animals that fall from rod for group those received methanolic extract (150 mg/kg) was 33.33%, which was equivalent to control group (33.33%), but % of animals fall from rod with in one min. for group those received methanolic extract was 50%, which was significantly more than that of control group.

MOTOR IN COORDINATION EFFECT OF ESSENTIAL OIL:

% of animals falls from rod with in one min. for group those received essential oil was 66.66%, which was significantly more than that of control group (33.33%).

HYPNOTIC ACTIVITY:

Hypnotic effect method based on potentiation of thiopental induced sleeping time by extract & essential oil was used to study the effect of plant materials[11, 12]. After 1 hrs. of administration of extracts & essential oil thiopental sodium (25 mg/kg, ip) was given to induce sleep. The interval between loss and recovery of righting reflex was used as index of hypnotic effect. The time interval between injection of thiopental and start of sleep was recorded as latency time. In the control group normal saline was injected (10 ml/kg i.p.), (Table 2).

HYPNOTIC EFFECT OF METHANOLIC EXTRACT:

Sleeping time in animal receiving 150 mg/kg of methanolic extract was increased to 13 ± 0.55 min. that was nonsignificantly more than that of control (9 ± 0.98 min.) and in those receiving 250 mg/kg of methanolic was increased to 15.46 ± 0.88 min. that was significantly different compared to control (Fig. 1).

The time interval between injection of thiopental and onset of sleep in all groups measured as latency time, methanolic extract in the dose of 150 mg/kg shortened the latency time of sleep to 3.945 ± 0.56 min. which was nonsignificantly less than that of control (4.91 ± 0.61 min.). The latency time by group those received 250 mg/kg methanolic extract shortened to 3.52 ± 0.33 min. again it was nonsignificantly different compared to control (Fig. 2). .

HYPNOTIC EFFECT OF ESSENTIAL OIL:

Sleeping time in animal receiving essential oil was increased to 23.98 ± 0.91 min. that was significantly more than that of control (9 ± 0.98 min.) (Fig. 1). The latency time by group those received essential oil shortened to 2.2 ± 0.33min, it was significantly different compared to control (4.91 ± 0.61 min.) (Fig. 2).

Present study demonstrated hypnotic effect for 250 mg/kg dose of methanolic extract & essential oil of fruits of *Zanthoxylum budrunga* W. Methanolic extract 150 mg/kg was not significantly comparable with control. Traditionally fruits of plant known to have stimulant effect as well as sedative activity, this type of activity profile can be compared with opium alkaloid which possesses stimulant effect at low dose initially & marked sedative effect after cumulative effect or after high dose. Since present study reveals that essential oil & extract of fruits of *Zanthoxylum budrunga* showed sedative activity, essential oil also known to have local anesthetic activity & in present study it shows potent sedative activity as compared to control, thus fruits of plant can be considered as potent CNS acting drug. Fruits contain several components such as flavones, alkaloids, saponins, linalol, pinene, citral, limonene, etc. the responsible component(s) for hypnotic effect of fruits of *Zanthoxylum budrunga* is uncertain to us and can not be concluded from the result of the present study. Alkaloids traditionally reported to have potent hypnotic effect in many plant species & saponins also have been reported to regulate sedative-hypnotic effect in plant like; *R. damascene*, etc[13]. therefore it can be suggested that these compounds may be responsible for hypnotic effect of methanolic extract of fruits of *Zanthoxyum budrunga* W. The potent hypnotic effect of essential oil of fruits of *Zanthoxylum budrunga* strongly supported by fact that it also been reported for local anesthetic action, but responsible compound(s) for activity can't be suggested on the basis of present study.

The result of present study indicated hypnotic effect of *Z. budrunga* which was comparable with significant difference to that of control (normal saline).

Table-1: Motor in coordination effect of methanolic extract & essential oil of fruits of Zanthoxylum budrunga.

Treatment	Dose	% of rota rod failed animals
Methanolic extract	(150 mg/kg body weight, i.p.)	33.33%
Methanolic extract	(250 mg/kg body weight, i.p.)	50%
Essential oil	(0.2 ml of 0.25 % oil in saline, i.p.)	66.66%
Control	(10 ml/kg body weight, i.p.)	33.33%

Table-2: Sedative-hypnotic activity of methanolic extract & essential oil of fruits of Zanthoxylum budrunga.

Treatment	Dose	Sleep duration Mean ± SEM (min.)	Onset of sleep Mean ± SEM (min.)
Methanolic extract	(150 mg/kg body weight, i.p.)	13±0.55	3.945±0.56
Methanolic extract	(250 mg/kg body weight, i.p.)	15.46±0.88	3.52±0.33
Essential oil	(0.2 ml of 0.25 % oil in saline, i.p.)	23.98±0.91	2.2±0.33
Control	(10 ml/kg body weight, i.p.)	9±0.98	4.91±0.61

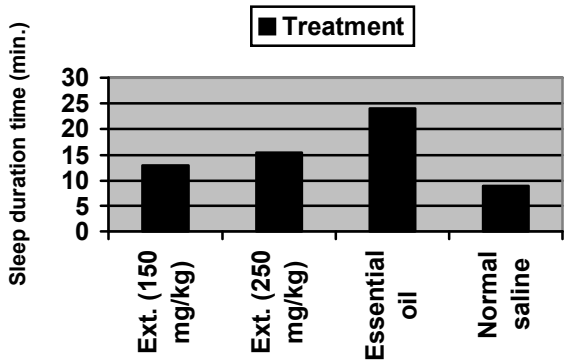

Fig. 1- Effect of methanolic extract in 150, 250 mg/kg dose & essential oil on thiopental-induced sleeping time in mice. [Data are presented as mean±SEM of 6 mice. Ext-Extract. ***p<0.01 compared to control].

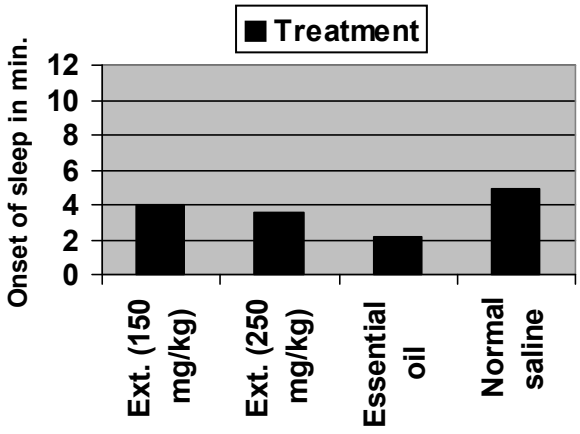

Fig. 2- Effect of methanolic extract in 150, 250 mg/kg dose & essential oil on the latency time of thiopental-induced sleeping time in mice. [Data are presented as mean±SEM of 6 mice. Ext-Extract. ***p<0.01 compared to control].

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