MALLA REDDY COLLEGE OF PHARMACY

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EVALUATION OF CNS STIMULANT ACTIVITY OF ETHANOLIC EXTRACT OF *PIPER LONGUM* LINN

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DEPARTMENT OF PHARMACOLOGY

UNDER THE GUIDANCE OF

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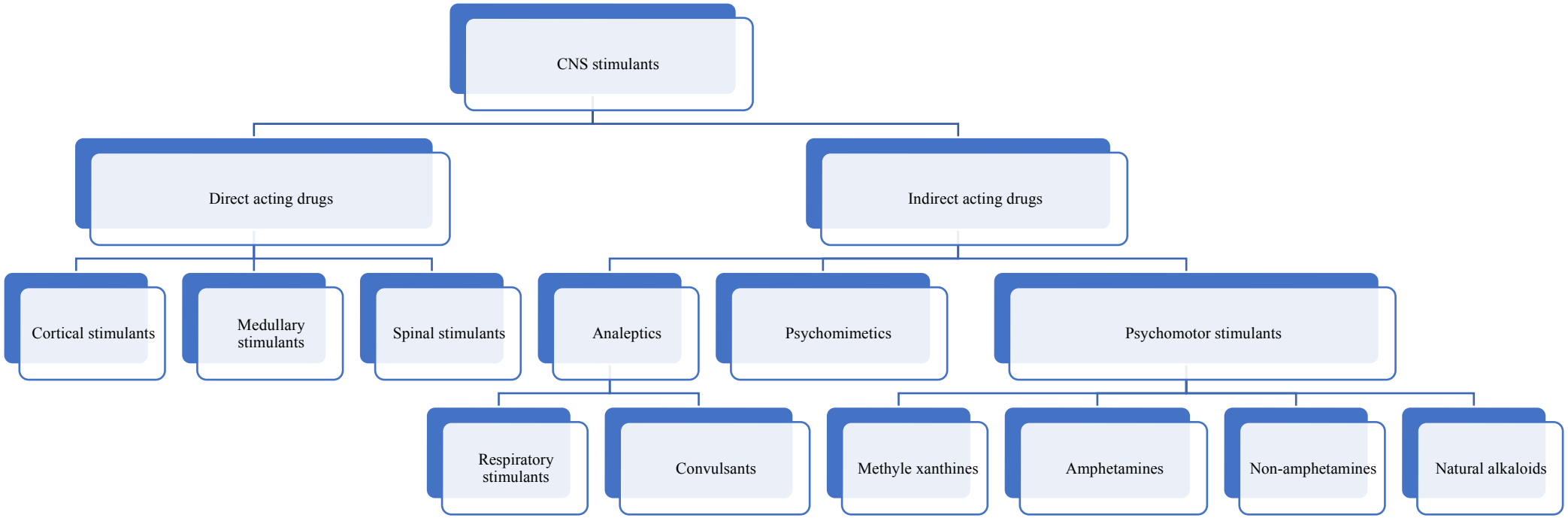
CONTENTS

INTRODUCTION

CNS stimulants

- They are drugs which increase the muscular (motor) and the mental (sensory) activities:
- Their effects vary from the increase in the alertness and wakefulness (as with caffeine) to the production of convulsion (as with strychnine) and sometimes lead to death in over dose



Piperlongum Linn, basically recognized as (Pipalli in India) Long Pepper and a representative of a Piperaceae family. It is distributed in West Bengal, Maharashtra, Eastern Uttar Pradesh, Madhya Pradesh, Kerala, Tamil Nadu, Karnataka, Andaman & Nicobar Islands and is recognized to have antibacterial qualities (Reddy et al., 2001; Yadav et al., 2020). P. longum is a valuable medicinal plant used to treat respiratory and digestive disorders, cancer, snake and insect bites and other ailments. The fruit comprises enormous alkaloids and chemically effective compounds, the first most prevalent is piperine and recent research revealed the content present in P. longum has anti-cancer properties and also antioxidant properties.

PLANT INTRODUCTION

Botanical name *: Piper longum* Linn
Common name : Indian long pepper
Local names : English: long pepper
Telugu: pippallu
Hindi: pipli

Taxonomic classification ; Kingdom: Plantae
Order: Piperals
Family: Piperaceae
Genus: *Piper*
Species: *longum*

CHEMICAL CONSTITUENTS

Piperine is the major and active constituent of long pepper (*Piper longum Linn*) the piperine content is 3.5% (on dry weight basis) in *piper longum Linn*

USES

It is used as a antioxidant, antifungal, anticancer, antimicrobial, antidepressant, analgesic, antibacterial.

AIM AND OBJECTIVES:

AIM: The aim of this study is to evaluate the Central nervous system stimulant activity in rats by Insilco models.

OBJECTIVES OF THE STUDY: Specific objectives of this research work involve in:

- Collection of fruit of piper longum Linn.
- Phytochemical analysis of the extract.
- Check the CNS stimulant activity of the fruit extract.
- To conduct CNS Stimulant activity by of the Actophotometer, Rotarod apparatus and Phenobarbitone induced sleeping time.

LITERATURE REVIEW

- **Anshuley Tiwari el al. (2020)** Reported the review article on the methods of isolation, purification and biological properties of piper longum. In which they have studied the different methods of extraction of the the plant drug and also they have studied the biological properties of the drug. They have concluded that the black pepper and long pepper are the important sources of piperine is being udsed in large amounts in traditional pharmacop9ia even today despite of long years of use and the best experimental models and highly refined molecular and cell biology research methods and tool;s available today the mode of action of piperine remains a mystery.

- **Bala murugan. K et al. (2020)** Reported the article on enhanced anti-depressant like activity of Diosgenin and silymarin in combination on pheno barbitone induced sleeping time in rats. In this research article diosgenin and silimarine caused significant decrease in the on set and duration of sleep in phenobarbitone 16 | P a g e induced sleeping time in rats when compared with control. In this study it was found that the administration of these drugs in combination gives better results.

- **Sreejai R et al. (2019)** Reported the comparative study of anti-microbial and phytochemical analysis of *piper longum*. The results obtained by this comparitive showed that the both plants species *P.longum* and P. nigram showed a high zone of inhibition against E.coli is very severe, so the acholic extract of *P.Nigrum* may be used against the gram negative E.coli and it is useful for the curing of the disease caused by the E.coli.
- **Devesh D gosavi et al. (2020)** Reported the spontaneous motor action of alcholic extract of withania coagulants fruits on the swiss albino rat. It is concluded that the ethanolic extract of the drug was able to increase the locomotory activity of the albino rat by this we can say that the drug has the CNS stimulant activity and CNS stimulants are also can increase the locomotory activity.
- **Jadhav et al. (2021)** Reported on the CNS stimulant activity of hydro alcoholic extract of Brasssica oleracea L.var.italica in laboratory animals. Hydroalcoholic extract of BOVLI florets was evaluated for CNS stimulant effect using locomotor activity and spontaneous motor activity through Y maze test apparatus. Initially were prepared. Preliminary phytochemical screening of hydroalcoholic extracts of B. oleracea revealed that presence of some active ingredients such as alkaloids, flavonoids, tannins, carbohydrates, glycosides, saponins, terpenoids and phenols. Acute oral toxicity study revealed the safety of the extract and further CNS stimulating activity observed by increased in locomotor and spontaneous motor activity in rats. Thereafter, the extract at higher dose shown significant increase in dopamine and serotonin level and affirmed its dose dependent potentiality as CNS stimulant.

MATERIALS AND METHODOLOGY

METHODOLOGY:

Extraction of piperine from *piper longum* (Soxhlet extraction)

Procedure:

•The apparatus was assembled.

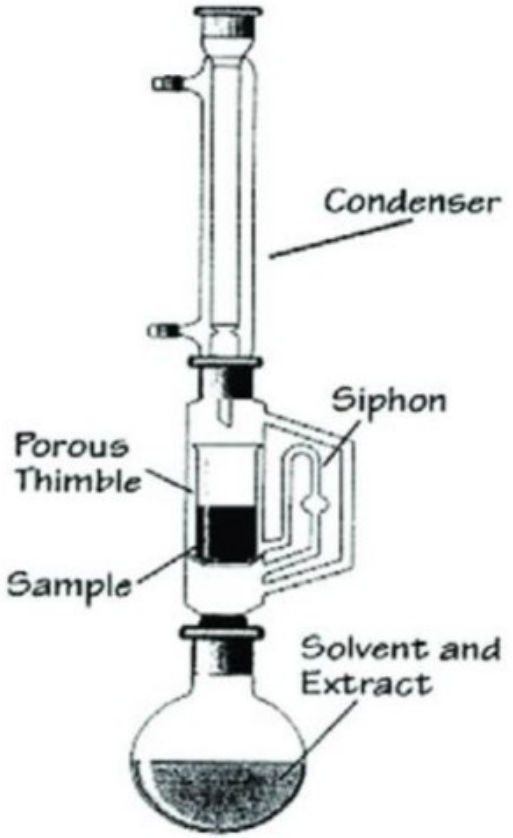
• Fill the round bottom flask with 450ml of solvent (ethanol).

• Put the thimble containing sample (30gm of power) into extraction tube.

• Attach the extraction tube with flask containing solvent.

• Attach a condenser unit with the extraction tube and run the water.

• Fix the Soxhlet apparatus on hot plate and heat the flask containing solvent at 70 degrees Celsius.



• The solvent starts to evaporate and falls in the extraction tube after condensing.

• Continue this process till all the drug is extracted.

• Discontinue the process and take out the thimble.

• Again, attach the extraction tube with flask containing solvent along with long pepper and condenser unit.

• Again, heat the flask to recover the solvent.

• Discontinue the process and clean the extraction tube and thimble.

PHYTOCHEMICAL ANALYSIS:

. Test for alkaloids:

Dragendroff's test:

- 2mg of extract +5ml od distilled water.
- 2M Hcl was added until an acid rxn occurs.
- 1ml dragendroff's reagent is added.
- Orange red ppt indicates the presence of alkaloids.

Test for alkaloids:

Hager's test:

- 2mg of extract +few drops of Hager's reagent.
- Yellow ppt gives the presence of alkaloids.

Wagner's test:

- 2mg of extract was acidified with 1.5%v\v of HCl and few drops wagner's reagent.
- Yellow or brown ppt indicates the presence of alkaloids.

Mayer's test:

- 2mg extract + Mayer's reagent
- White or pale ppt indicates the presence of alkaloids.

- Test sol. With few drops of lead acetate (10%) solution gives yellow ppt.

Test for flavonoids:

Ferric chloride test;

- Test sol. + few drops of fecl3 gives intense green color.
- Zinc chloride acid reduction test:
- Test solution with zinc dust and few drops of hcl shows magenta red color.

Lead acetate test:

- Test sol. With few drops of lead acetate (10%) solution gives yellow ppt.

Test for saponins

Foam test:

- To the extract solution add a drop of sodium bicarbonate.
- The test tube was shaken vigorously for 3min.
- Formation of honey comb like forth indicates the presence of saponins.

Test for tannins:

Ferric chloride test:

- 1-2ml of extract, few drops of 5% w\v fecl3
- A green color indicates the presence of pseudotanins.

Gelatin test:

- Test solution when treated with gelatin solution gives the white ppt.

INSILCO STUDIES ON RATS

- ## Actophotometer

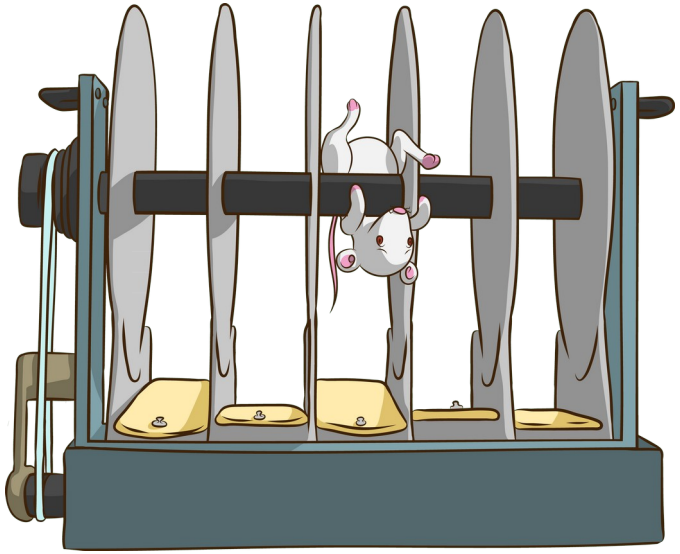
Procedure:

- Animals were weighed and numbered
- Equipment was turned on and all the rats were placed on the equipment each one for 10 minutes and the reading was recorded.
- Inject diazepam and after 30 min retest each mouse for activity scores for 10 min. Note the difference in the activity before and after Diazepam
- Calculate percent decrease in motor activity.

- ## Rota rod apparatus

Procedure:

- Weigh the animals and number them.
- Turn on the instrument select an appropriate speed (20-25rpm)
- Place the animal one by one on the rotating rod. One can place more than one mouse. Note down the fall off time when the mouse falls from the rotating rod. A normal mouse generally falls off within 3-5 min
- Inject diazepam to all animals. After 30 min repeat the experiments as done in step note the fall off time.
- Compare the fall off time of animals before and after diazepam treatment.



PHENOBARBITONE INDUCED SLEEPING

Procedure:

1. Weigh and number the animals.
2. Divide them into two groups, each comprising of at least 6 mice.
3. To the first group administer distilled water + phenobarbitone,
4. To second group inject Sleep inducer (Phenobarbitone)+ Caffeine drug in
5. Note the onset and duration of sleep due to phenobarbitone in both the groups.

RESULTS

SL. NO	PHYTOCHENICAL	OBSERVSTION
1	ALKALOIDS	+
2	SAPONINS	+
3	CARBOHYDRATES	+
4	TANNINS AND PHENOLS	+
5	FLAVONOIDS	-
6	FIXED OILS AND FATS	+
7	LIGNIN	+

Note '+'shows the presence of phytochemical '- 'shows the absence of phytochemical.

Controlled group	Drugtreated (diazepam)	Percentage
Locomoter index	Locomoter index	Locomoter index
167	77	46.10%
167	167	100%
167	57	34.13%
167	167	100%
138	67	48.55%
177	15	8.47%

Actophotometer readings

Group selected	Readings	Mouse no.1	Mouse no.2	Mouse no.3	Average
Vehicle Treated	Fall off time (Sec)	52	50	53	52.33
Drug treated (diazepam)	Fall off time (Sec)	42	39	45	42

Rata rod apparatus reading

Mice and treatment	Distilled water + phenobarbitone treated	Sleep inducer (phenobarbitone)+ drug treated
	Duration of sleeping minutes	Duration of sleep-in minutes
Mice -1	25	15
Mice -2	24	17
Mice -3	26	16
Mice -4	25	17
Mice -5	25	16
Mice -6	24	16

Phenobarbitone induced sleeping readings

DISCUSSION

- The percentage yield of ethanolic extract of Piper longum Linn with respect to the dried powder was found to be 10%.
- Preliminary Phytochemical screening of the fruit extract of Piper longum Linn revealed the presence of alkaloids, saponins, tannins, carbohydrates, fixed oils and fats are present.
- By observing the actophotometer readings we can see that the locomotory index of the animals have been decreased after the administration of the drug. So we can say that the diazepam is CNS depressant.
- By observing the rota rod apparatus readings we can see that the fall of time is decreased after the adminstration of the diazepam. So we can say that the drug is acting as the skeletal muscle relaxant.
- By the observation experiments results of phenobarbitone induced sleeping we can see that the duration of sleep of the rat was decreased as the drug was injected to the rat. we can say that the drug which has been injected to the rat acted as a stimulant

CONCLUSION

The ethanolic extract of *Piper longum* Linn posses significant CNS stimulant activity. The present study indicated the presence of phytochemical constituents that are responsible for CNS stimulant activity.

Future insights

The extract further is subjected to invitro and in vivo models to confirm the CNS stimulant activity.

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THANK YOU