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Central Nervous Depressant Activity of Piperine

Won Sick Woo, Eun Bang Lee and Kuk Hyun Shin

Natural Products Research Institute, Seoul National University, Seoul 110, Korea

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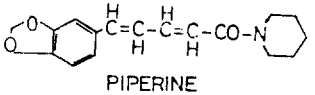
Abstract□ Piperine showed a central nervous system depressant activity which was characterized by the antagonistic effect against chemoshock seizure as well as potent muscular incoordination in mice.

Keywords□ Piperine—acute toxicity—strychnine mortality—pentetrazole seizure—maximal electroshock seizure—rotarod test.

The methanolic extract of the fruits of *Piper retrofractum* was reported to show considerable elongation of hexobarbital induced sleeping time and antagonism against strychnine mortality in mice [1]. On the basis of the result, piperine, an active principle, was isolated from the plant and it was preliminarily disclosed that its pharmacological activity was a central nervous system depressant activity in mice[2].

Contrary to our results, Singh *et al.* reported that piperine exhibited an analeptic activity, *i.e.* increases in the ED_{50} (hypnotic response) and LD_{50} of pentobarbital sodium in mice[3].

In order to clarify such ambiguity, further pharmacological study of piperine was carried out. The chemical structure of piperine is shown below.



PIPERINE

($C_{17}H_{19}NO_3$ = 285.33, mp 130°)

EXPERIMENTAL

Materials and Animals

Piperine, mp 130°, isolated from *Piper retrofractum* and recrystallized from ethanol, is a white crystalline substance, insoluble in water and other physico-chemical data are in agreement with the data[4,5]. This substance was identical with commercial sample supplied by Sigma Co. (mmp, IR, UV, NMR, coTLC). Strychnine nitrate and pentetrazole were purchased from Shionogi Pharm. Co. Ltd. and Tokyo Kasei Co. Ltd., Japan, respectively. Phenytoin and chlormezanone were of USP grade. All the samples were suspended in 0.5% CMC solution for the administration to animals. Male dd mice weighing 18 to 24 g of body weight were used throughout the experiments.

The Acute Toxicity

Following administration of the sample to groups of 8 animals, the median lethal dose was evaluated on the 3rd day. The intraperitoneal LD_{50} was calculated by probit method and the oral LD_{50}, by Up and Down method.

Strychnine Mortality Test

The convulsive death was induced by subcutaneous injection of 1.3 mg/kg of strychnine. Graded doses of piperine was administered intraperitoneally to each group of 6 to 8

animals 30 min prior to the administration of strychnine. The ED_{50}, a dose of the sample which protected 50% of mice against strychnine mortality was calculated by probit method.

Pentetrazole Induced Convulsion Test

Pentetrazole was injected subcutaneously at a dose of 85 mg/kg to mice according to the method of Swinyard *et al.*[6] Graded doses of the samples were injected to each group of 6 to 8 animals intraperitoneally 30 min or orally 60 min prior to pentetrazole administration.

The ED_{50}, a dose of the sample which protected 50% of mice against the tonic extensor phase of convulsion was calculated by probit method.

Maximal Electroshock Seizure Test

Maximal electroshock seizure in mice was induced by supramaximal alternating current (60 cycle, 50mA) delivered through corneal electrodes for 0.2 sec. The electroshock apparatus and the method were employed according to the description by Woodbury and Davenport[7]. The samples were administered 60 min prior to giving the shock. The ED_{50}, a dose of the sample which protected 50% of mice against the seizure was calculated by probit method.

Rotarod Test

A method of Dunham and Miya[8] was used and the mice which persisted more than 1 min on the rotating rod(4cm in diameter, 10 revolutions/min) were used for the test. The sample was pretreated intravenously 30 min or orally 60 min prior to the test, and the mice which were dropped within 1 min due to sample treatment were recorded. The ED_{50}, a dose of the sample that caused 50% of mice to drop was calculated.

RESULTS

The Acute Toxicity

The LD_{50} values of piperine were 287.1 mg/kg intraperitoneally and 1636.8 mg/kg orally as shown in Table I. The behavioral changes of staggering gait, muscular relaxation and ataxia in mice could be observed within 60 min after dosing near the LD_{50} levels of piperine and loss of righting reflex was

Table I: Acute toxicity of piperine

Animal	Administration route	LD_{50} (mg/kg)
Mouse (M)	i.p.	287.1 (227.5–331.9)[a]
Mouse (M)	p.o.	1636.8[b]

a) Probit method
b) Up and down method
Numbers in parentheses indicate the 95 % confidence limit.

Table II: Protective evaluation of compounds in convulsion

Compound	Administration route	scStM ED_{50}* (mg/kg)	scPT ED_{50}* (mg/kg)	MES ED_{50}*** (mg/kg)
Piperine	i.p.	11.5(5.8– 15.0)	22.4(11.7– 36.4)	>200
Phenytoin	i.p.	Not tested	Not active	20.2(14.0– 30.5)

* scStM ED_{50}, Subcutaneous strychnine (1.3mg/kg) mortality test, median effective dose.
** scPT ED_{50}, Subcutaneous pentetrazole (85mg/kg) seizure threshold test, median effective dose.
*** MES ED_{50}, Maximal electroshock seizure (50mA, 0.2 sec.) test, median effective dose.
Numbers in parentheses indicate the 95% confidence limits.

Table III: Protective evaluation of compounds in convulsion and rotarod test

Compound	Administration route	scPT ED_{50}* (mg/kg)	MES ED_{50}** (mg/kg)	RR ED_{50}*** (mg/kg)
Piperine	p.o.	211.5(82.7–402.5)	>1300	89.1(63.2–125.7)
Phenytoin	p.o.	Not active	16.8(9.8– 31.6)	489.8(360.1–666.1)
Chlormezanone	p.o.	14.9(6.6– 39.3)	197.3(169.5–220.7)	129.7(90.0–185.4)

* scPT ED_{50}, Subcutaneous pentetrazole seizure threshold test, median effective dose.

** MES ED_{50}, Maximal electroshock seizure test, median effective dose.

*** RR ED_{50}, Rotarod test, median effective dose.

Numbers in parentheses indicate the 95% confidence limits.

eventually induced.

Effects on Pentetrazole Induced Seizure

Piperine showed protective effect against pentetrazole seizure, as shown in Table II and III. The ED_{50} values were 22.4 mg/kg intraperitoneally and 211.5 mg/kg orally. Chlormezanone, a muscle relaxant tranquilizer, showed potent protection against the seizure. However, phenytoin, an antiepileptic, was not active.

Effects on Maximal Electroshock Seizure

Piperine showed only very weak protection against maximal electroshock as shown in Table II and III. The ED_{50} values are above 200 mg/kg and above 1300 mg/kg in intraperitoneal and oral administration, respectively. The oral ED_{50} values of phenytoin and chlormezanone were 16.8 and 197.3 mg/kg, respectively.

Effects on Responses of Rotarod Test

Piperine caused heavy muscular incoordination in rotarod test as shown in Table III. Its oral ED_{50} value was as small as 89.1 mg/kg and the values for phenytoin and chlormezanone were 489.8 and 129.7 mg/kg, respectively.

DISCUSSION AND CONCLUSION

Piperine showed a slight antielectroshock activity but a marked antichemoshock activity and caused muscular incoordination in mice. Piperine exhibited the protective effects against strychnine and pentetrazole seizures markedly by intraperitoneal administration and the therapeutic indices, LD_{50} ip/ED_{50} ip were as large as 25 and 12.8, respectively.

Oral pretreatment of piperine also had the protective effect against pentetrazole seizure and the therapeutic index, LD_{50} po/ED_{50} po, was as large as 7.7, whereas, a more potent effect was shown in the rotarod test; the index, LD_{50} po/ED_{50} po, was 18.4. However, piperine could not protect the mice from the electroshock seizure at dosse of 200 mg/kg ip and 1300 mg/kg po.

Phenytoin used as a reference antiepileptic agent showed much more potent antielectroshock activity, whereas showed no antichemoshock. This result was in agreement with that of Krall *et al.*[9] Chlormezanone used as a muscle relaxant tranquilizer showed more potent antipentetrazole activity compared with antielectroshock.

Krall *et al.*[9] have evaluated central nervous depressant drugs for the protective effect against pentetrazole and maximal electroshock seizure, and classified them into two groups:

(1) drugs with more potent protective activity against electroshock seizure, (2) drugs with less potent activity against pentetrazole seizure than against electroshock seizure.

The drugs belonging to the first group were chlorpromazine, meprobamate, metharbital, ethusuximide, trimethadione, phenobarbital, etc. and the drugs belonging to the second group were primidone, amitriptyline, imipramine, phenytoin, etc. According to this classification, piperine and chlormezanone belong to the first group.

The rotarod test has been carried out in order to evaluate muscular incoordination. Pretreatment of piperine caused 2.4 times more marked muscular incoordination than the protective effect against pentetrazole seizure. Phenytoin showed relatively low effcet in the rotarod test as expected and it is evidence that mice would be capable of normal coordinated movement while under the influence of the drug. While chlormezaooe showed more potent activity in the rotarod test compared with antielectroshock activity. This result seems to be associated with the muscle relaxant activity of chlormezanone. Having most potent activity in the rotarod test, piperine is considered to be not appropriate as an anticonvulsant, but expected to be used as a muscle relaxant. A detailed evaluation for its muscle relaxant activity is in progress.

Singh *et al.*[3] reported pharmacological activities of piperine. Its LD_{50} values in mice were 31.2 mg/kg intraperitoneally and 56.2 mg/kg orally. Each of these values corresponded to be 9.2 and 29 times more toxic in comparison with the data obtained in the present study. Furthermore, it has been described that piperine sample was isolated from the fruits of *P. longum* and it had central nervous stimulant activity and antagonistic effect against pentobarbital hypnosis and lethality in mice.

Our experimental results clearly demonstrated that piperine had central nervous system depressant activity, characterized by the antagonistic effect against central nervous stimulants such as strychnine and pentetrazole. Though these discrepant findings are not explainable at present, it might be assumed that the sample of Singh *et al.* could not be piperine.

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