



International Journal of Pharmacology & Toxicology

www.ijpt.org

EFFECT OF ETHANOLIC EXTRACT OF *PIPER NIGRUM* L. FRUITS ON MIDAZOLAM INDUCED HYPNOSIS IN RATS

*Md.Gayasuddin, Md.Parvez, Mohd Iqbal, G.Venkataiah

Smt. Sarojini Ramulamma College of Pharmacy, Palamuru University, Mahaboobnagar, Andhra Pradesh, India.

ABSTRACT

Although herbal medicines are generally perceived as safe when used alone at the recommended dose and duration, there are increasing evidences of herb-drug interaction which may lead to serious adverse reactions or failure of therapy with conventional medicines. The aim of this study was to investigate the effect of ethanolic extract of *Piper nigrum* L. fruits on Midazolam (substrate for CYP3A4) induced hypnosis. In this study we investigated the effect of subchronic treatment of 100mg/kg of extract p.o. for five consecutive days on an i.p. hypnotic dose (50mg/kg) of Midazolam in rats (n=5). Sleeping time is significantly increased in extract pretreated group to 35% to that of control value of Midazolam induced hypnosis without affecting the sleep latency. The current study demonstrates that ethanolic extract of *Piper nigrum* L. fruits might have inhibited metabolism of the drug by inhibiting CYP3A4 as the efficacy of the drug is increased.

Keywords: *Piper nigrum* L., Midazolam, Hypnotic Indices.

INTRODUCTION

Now a day, the concurrent administration of herbal drugs and conventional medicines is more often seen. The close look at the possible drug-food/herb interactions is necessary to avoid side effects of the drugs [1,2].

Black Pepper is the dried fruit of *Piper nigrum* L., which is used in many herbal formulations and also used in food in view of its spicy taste as dietary supplement [3]. The main chemical constituent of *Piper nigrum* L. is the alkaloid piperine (a trans-trans isomer of 1-piperoyl piperidine), which has many pharmacological properties [1,2]. It is used for the treatment of diarrhoea and constipation in ancient times till present. Recent studies revealed that it has many other effects of pharmacological importance including, antimicrobial, antioxidant, and mainly as bioenhancer which promotes the absorption of many drugs and nutritional supplements. The bioenhancing activity is due to its inhibitory activity of drug metabolizing enzyme CYP3A4 [3-9]. Midazolam

is a short-acting benzodiazepine and has the anxiolytic, sedative, hypnotic, anticonvulsant, muscle relaxant and amnesic effects [10]. Midazolam is extensively used as hypnotic, pre-anaesthetic medicine and as post operative medicine for its amnesic property [10]. Midazolam is extensively metabolized in both the liver and the intestine via CYP3A4 and CYP3A5 to 1-hydroxyMidazolam and 4-hydroxyMidazolam which undergo rapid conjugation with glucuronic acid and then excreted into the urine within 24 h [10]. Many drugs and herbs have been reported to cause pharmacokinetic drug interaction with Midazolam by the inhibition of CYP3A4, such as curcumin [11], itraconazole [12], DHA [13], green tea [14], *androgarphis paniculata* [21], diltiazem and verapamil [15,16], which can modulate efficacy of Midazolam and may increase the risk of side effects such as drowsiness, confusion, memory loss, hypotension or respiratory depression.

After administration of hypnotic dose of the drug it produces immediate or delayed action and also that it induces sleep which lasts at a particular time relating to

Corresponding Author:- **Md. Gayasuddin** Email:- ghayassrcp@gmail.com

the duration of action of the drug [12,15]. The time elapsed between the administration of drug and the onset of losing righting reflex said to be as sleep latency and the interval between the loss and recovery of the righting reflex was said to be as the sleeping time in an animal model [12,15]. In this study the effect of subchronic treatment of ethanolic extract of *Piper nigrum* L. fruits on the Midazolam induced sleep model in rats was studied.

MATERIAL AND METHODS

Experimental Animals

Wistar albino rats (150–200 g) of male were purchased from Sainath Agencies, 1-6-197/45/D, Bapujinagar, Musheerabad, (REG.No282/99/CPCSEA), Hyderabad, India and Maintained under standard environmental laboratory conditions and fed with laboratory diet and water *ad libitum*. All the experimental procedures had been approved by the Institutional Animal Ethical committee (IAEC) of Smt. Sarojini Ramulamma College of Pharmacy (R. No: 51/01-CPCSEA/2012/19).

Drugs and Chemicals

Midazolam (Mezolam^R an injection containing 5mg/ml of Midazolam of Neon India) and Diazepam (Calmpose^R an injection containing 5mg/ml of Diazepam of Ranbaxy India) kindly supplied by Healthcare Pharma Distributors, Mahbubnagar were used.

Piper nigrum L. fruits

Piper nigrum (L) fruits of high quality were obtained from local commercial sources, Authenticated by botanist Dr. Madhava Chetty, Assistant Professor, Department of Botany in S.V. University, Tirupathi, Specimen number (1011).

Preparation of ethanolic extract of *Piper nigrum* L. fruits

Fruits were coarsely powdered and extracted with 95% ethanol in a soxhlet extractor for 8 hours. The solution is filtered and concentrated on a water bath at 60°C. 10mL 10% alcoholic KOH solution is added and after a while decanted from the insoluble residue. The alcoholic solution is left overnight, whereupon yellow needles are deposited. Yellow needles were recrystallised using ethanol. Practical yield is calculated to be 10.8 % w/w [17].

Phytochemical screening

Phytochemical screening of the crude extract was carried out employing standard procedures [18,19]. It revealed the presence of chemical constituents such as alkaloids, glycosides, flavonoids, tannins, terpenes, saponins, carotenoids, phytosterols and fixed oils.

Experimental design

Effect of ethanolic extract of *Piper nigrum* L. fruits on Midazolam induces hypnosis

Healthy male wistar albino rats were divided randomly in three groups of 5 rats in each group. Test group is gavaged (16-gauge gavage needle) with 100mg/kg (1% Tween 80 as vehicle) of ethanolic extract of *Piper nigrum* L. fruits for 5 consecutive days followed by Midazolam 50mg/kg i.p. 1 hour after the fifth day dose of the extract. Tween 80 was added to the vehicle to produce a fine suspension of the extract and accurate dosing. Rats in control group and standard (Midazolam alone) groups were similarly gavaged with vehicle equivalent volume (about 0.5ml) of the 1% Tween 80 (taking into consideration reports that suggest Tween 80 to have inhibitory effects on the function of P-gp and CYP3A4 [23,24] for 5 days followed by water for injection i.p and Midazolam 50 mg/kg i.p. respectively. After Midazolam administration hypnotic indices were recorded by observers blind to the treatment schedule. The time elapsed between the administration of Midazolam and the onset of losing righting reflex was recorded as sleep latency and the interval between the loss and recovery of the righting reflex was recorded as the sleeping time. The whole experiment was carried in a quite room maintaining the room temperature 22±2°C.

Statistical analysis

Statistical analysis of the data was done using version 5.0 of "Prism Graph pad". All the values of the experimental results were expressed as mean ± SD and analyzed by one way ANOVA followed by "Dunnett's Test comparison test".

RESULTS AND DISCUSSION

The efficacy of Midazolam i.p hypnotic dose (50mg/kg) to produce hypnotic effect i.e. sleep latency and sleeping time is measured [12,15].

After administration of hypnotic dose of Midazolam, the sleeping time significantly increased in extract pretreated group when compare to Midazolam alone treated group, whereas the sleep latency does not affected by the extract pretreatment.

Data suggests that piperine an abundant alkaloid present in *Piper nigrum* L. fruits is a potent inhibitor of CYP3A4, and Midazolam is a CYP3A4 substrate which indicates a chance of a potential herb/food-drug interaction [7,10,18]. The increase in the sleeping time may be due to decrease clearance of the drug which intern may be due to inhibition of metabolism of Midazolam [17,19].

Hence there is need of attention to be taken while co-administration of Midazolam with herbal formulations containing *Piper nigrum* L. fruits or its extract, which ever, since, the dose of the extract used in this study is similar or less than that is supplied by the prescription in herbal medicine [20-24]. However the dose administered

of the extract is more than the daily consumption of the *Piper nigrum* L. fruits in diet [25].

Midazolam is a sedative/hypnotic/ drug often used as pre-anaesthetic medication to reduce anxiety and as post-operative medicine for its amnesic property [10].

Hence, there is need of attention to be taken when Midazolam is to be used, and concurrent use of herbal formulations containing black pepper fruits should be taken into consideration, as it may interact with the drug and the dose of Midazolam may prove wrong.

Table 1. Sleep latency of single i.p. dose of Midazolam with and without pretreatment of extract, control group is administered i.p. with equivalent volume (about 2ml) of water for injection I.P.

Group	Rat number					Sleep latency (minutes)
	1	2	3	4	5	
Standard	5	4	4.5	5	4.5	4.6±0.41
Test	4.5	5	5	4.5	5	4.8±0.27 ^{ns}

Values are mean±SD; n=5, ns-non significant

Table 2. Sleeping time of single i.p. dose of Midazolam with and without pretreatment of extract, control group is administered i.p. with equivalent volume (about 2ml) of water for injection I.P.

Group	Rat number					Sleeping time (minutes)
	1	2	3	4	5	
Standard	65.0	70.5	48.0	72.5	85.0	68.2±13.45
Test	93.5	89.0	104.0	93.0	80.5	92.0±8.49 ^{****}

Values are mean ± SD; n=5, ****p<0.0001

Table 3. Mean hypnotic indices (sleep latency and sleeping time) of single dose of i.p. administered Midazolam 50mg/kg with and without subchronic p.o. pretreatment of extract in rats (n=5), control group is administered i.p. with equivalent volume (about 2ml) of water for injection I.P.

Hypnotic Index	Standard	test
Sleep Latency (min)	4.62±0.418	4.8±0.273 ^{ns}
Sleeping Time (min)	68.20±13.46	92.0±8.493 ^{****}

Values are mean ± SD; n=5, ****p<0.0001, ns-non significant.

Fig. 1. Effect of ethanolic extract of *Piper nigrum* L. fruits pretreatment on sleep latency of Midazolam induced sleep in control, standard and test groups (n=5), ± SD errors.

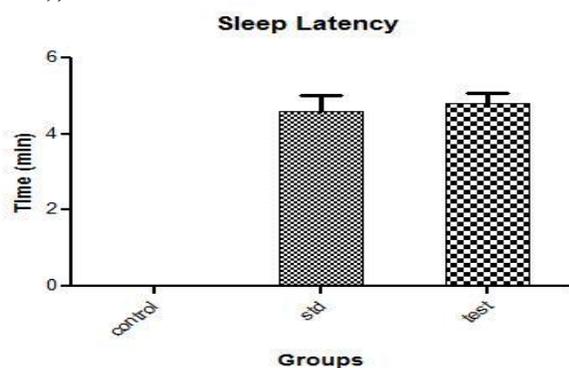
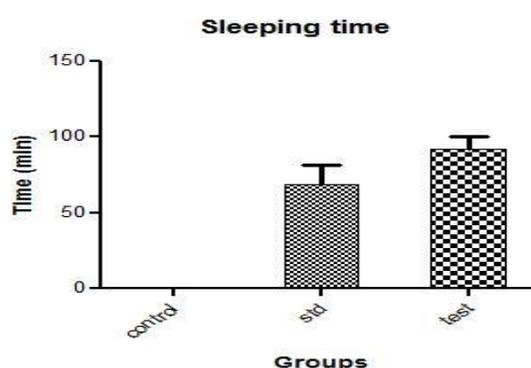


Fig. 2. Effect of ethanolic extract of *Piper nigrum* L. fruits pretreatment on Midazolam induced sleeping time in control, standard and test groups (n=5), ±SD errors.



CONCLUSION

In conclusion, this study provided evidence to show that *Piper nigrum* L. fruits extract modestly increases the efficacy of Midazolam, probably due the inhibition of the metabolism of Midazolam mediated by

CYP3A4. Significant increase in sleeping time of i.p. administered drug is due to the inhibition of its metabolism and may be due to decrease in the clearance from the body that may result in unnecessary excessive sedation.

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