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

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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], andrographis 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
the duration of action of the drug [12,15]. The time
eclapsed 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® an injection containing
5mg/ml of Midazolam of Neon India) and Diazepam
(Calmpose® 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 eclapsed 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-anesthetic 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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Rat number</th>
<th>Sleep latency (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Standard</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Test</td>
<td>4.5</td>
<td>5</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Rat number</th>
<th>Sleeping time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Standard</td>
<td>65.0</td>
<td>70.5</td>
</tr>
<tr>
<td>Test</td>
<td>93.5</td>
<td>89.0</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Hypnotic Index</th>
<th>Standard</th>
<th>test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep Latency (min)</td>
<td>4.62±0.418</td>
<td>4.8±0.273ns</td>
</tr>
<tr>
<td>Sleeping Time (min)</td>
<td>68.20±13.46</td>
<td>92.0±8.493****</td>
</tr>
</tbody>
</table>

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.
REFERENCES