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The central nervous system depressant activities of Mycotoxin MT81 and its Acetylated and Benzoylated analogues

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Abstract: Mycotoxin MT81 isolated from *Penicillium nigricans* and its two structural derivatives viz. Acetylated MT81 (AcMT81) and Benzoylated MT81 (BzMT81) show antimicrobial activities as well as cause hepatotoxicity and nephrotoxicity. Present study deals with the CNS depressant activity of the above said toxins. Sedative-hypnotic and hypothermic actions were assessed by injecting MT81, AcMT81, and BzMT81 at three different doses prior to the administration of diazepam, chloropromazine and pentobarbitone respectively. The analgesic actions were studied by hot plate method. The sleep induced by diazepam, chlorpromazine and pentobarbitone were prolonged following the administration of MT81, AcMT81 and BzMT81. Significant hypothermia produced in a dose-dependent manner after the treatment with MT81 and its two derivatives. The toxins also potentiated the analgesic action of morphine significantly ($p \leq 0.001$). Being less toxic than the parent toxin MT81, the structural analogues showed more prominent analgesic activities, sedative effects and hypothermic actions.

Keywords: CNS depressant activity, Sedative, Hypnotic, Hypothermic, Analgesic.

Introduction

A large number of compounds, drugs are available which depress the central nervous system (CNS) and hypotonic effects (1, 2). In smaller doses many of these drugs can produce a state of drowsiness, and when used in this manner they are referred to as sedatives. A sedative compound decreases activity, moderates excitement and calms the recipient when used in larger doses; hypnotics may produce anesthesia, poisoning and death. These progressive dose-related effects may be indicated as follows:

Sedation= Hypnosis=Anesthesia = Coma = Death

The sedatives and hypnotics are used to allay nervousness, to induce sleep, if pain is absent and control convulsions. The hypnotics suppress cerebral activity sufficiently to blunt the patient awareness of the environment thereby establishing conditions favorable for sleep.

The general action of the hypnotics and sedatives is that of the depression of the CNS, which begins with the cortex and descends with increasing dosages to medullary centers. Certain compounds act at different points in the cortex and give the best therapeutic effect. Combination of the sedative with an analgesic often gives a synergistic effect in cases where pain and excitement co-exist. The hypnotics and

sedatives are usually classified into two categories: the barbiturates and nonbarbiturates. Barbiturates reduce cerebral activity, which again reduces the cerebral metabolic rate probably by activating chloride channels and potentiating GABA's effects on these channels. Protection of the brain against hypoxia might theoretically occur by this mechanism, by vasoconstriction or by inhibiting calcium or glutamate (3).

Mycotoxin MT81 was isolated, purified and identified in our laboratory from a locally isolated fungal strain of *Penicillium nigricans* (patent no. 156916 dated 15.2.82, Govt. of India). MT81 is a dextrorotatory polyhydroxyanthraquinone compound having molecular formula of $C_{22}H_{18}O_7$ and molecular wt. of 394(4). Its LD_{50} value is 35.1 mg/Kg body wt. in mice. MT81 is a good hyperglycemic (5), antimicrobial (6) and antileishmanial (7) agent. It produces massive bone marrow depression (8), liver (9), and brain (10), kidney (11) dysfunction.

To generate more potent and less toxic toxin, two structural analogues – Acetylated MT81 (AcMT81) and Benzoylated MT81 (BzMT81) were synthesized in our laboratory having LD_{50} values 87.10 and 44.66 mg/kg body weight in mice respectively. These two analogues were reported to possess antimicrobial (6) effects.

MT81, Acetylated MT81 and Benzoylated MT81 (BzMT81) all potentiate the hypnotic effect of barbiturate in the mouse and this property may be regarded as an expression of sedative action (12). It seemed possible that some compounds might prolong the effects of certain hypnotics by affecting vascular mechanism (13), for example, the absorption of the drug from the site of injection, its penetration into the 'blood-brain barrier', or its breakdown or excretion. Due to the proliferation of a granular endoplasmic reticulum (GER) in the cytoplasm of tubules of kidney, degeneration of mitochondria, and the reduction of P-450 content in the liver by many chemicals prolonged sleeping time in mice (14). It has been shown that drugs which increase brain 5-hydroxytryptamine (5-HT) usually increases sleep, whereas drugs which decrease brain 5-HT, induce a state of permanent wakefulness in mice (15,16). Lowered body temperature has been shown to prolong pentobarbitone anesthesia (17-19). The hypothalamic action is produced by dopaminergic mechanism in the central nervous system (20, 21). Various agents that lower body temperature, such as histamine, antihistamine and pethidine (22) also potentiate barbiturate anesthesia (18, 23). The CNS depressant agent is generally associated with hypothermia and reduction in the norepinephrine content; reduction in the norepinephrine content is also associated with analgesic action. MT81 and its structural analogues are responsible for the reduction of norepinephrine content of the brain and so it is highly probable that MT81 and its structural analogues may potentiate the analgesic action of some standard reference drugs. The present investigation is to study the sedative, hypnotic and analgesic actions of MT81, AcMT81 and BzMT81.

Materials and Methods:

Chemicals and reagents

Diazepam (Calm pose, Ranbaxy Lab., India), pentobarbitone sodium or Nembutal, Chlorpromazine hydrochloride (Largactii, May and Baker, India), Morphine (Govt. Opium and Alkaloid Works, Gazipur, India), Acetylated MT81 (Ac MT81) and Benzoylated MT81 (BzMT81) were synthesized in our laboratory and clinical thermometer with Fahrenheit scale (Hicks, India).

Animals

Male Albino (Swiss) mice weighing 20-25g (4-5 weeks old) were used. The animals were fed standard pellet diet and given tap water *ad libitum*.

Study of CNS depressant action

The experiments were performed in a quiet room with an ambient temperature of 22 ± 2^0 C. The mice were placed in a specially designed cage, one hour before the start of the experiment so as to acclimatize them to the environment. The experiments were performed between 12-18 hours each day to avoid behavioral changes resulting from circadian rhythm. The criterion for sleep in mice was loss of righting reflex. The mice were regarded as sedated when they were calm, immobile and all behavioral activities abolished while retaining their righting reflex for at least 5 minutes. The end point was the moment when the mice were placed on their backs, were able to right themselves three times consecutively.

Rectal temperature in mice containing only saline, PG, MT81, AcMT81 and BzMT81 respectively of sedative hypnotic parametric measurement were measured by inserting the clinical thermometer (about 2 cm) in the rectum at 15, 60, 120 and 240 minutes respectively after injection.

The analgesic actions were studied by hot plate method (24). The effects were observed at 15 and 30 minutes after intraperitoneal (i.p.) administration of the drugs. The reaction time was taken as the interval extending from dropping the animal on the hot surface until the instant the animal licked its hind paws. Maximum observation period was 2 minutes to avoid tissue injury. All other signs of discomforts such as kicking and dancing were disregarded. The temperature of the bath was maintained at $55^0\pm0.5^0$C.

Design of the experiment

Each mouse was used once only. The mice were divided into 62 groups, each group containing 10 mice only.

l*) For sedative- hypnotic and hypothermic action:*

Group1: Vehicle control (5 ml kg^{-1}, propylene glycol);

Group 2: Normal saline (D.B%, u/v; 5 ml kg^{-1});

Group 3: Diazepam (3 mg kg^{-1}, dissolved in propylene glycol);

Group 4: Chlorpromazine (10 mg kg^{-1}, dissolved in normal saline);

Group 5: Pentobarbitone (40 mg kg^{-1}, dissolved in 5 ml normal saline);

Groups6-8: MT81 at doses of 4.5, 6.0 and 9.0 mg kg^{-1} respectively dissolved in propylene glycol

Groups 9-17: MT8I at doses of 4.5, 6.0 and 9 mg kg^{-1} respectively injected one to each group 30 minutes prior to the administration of either diazepam (3 mg kg^{-1}) or chlorpromazine (10 mg kg^{-1}) or pentobarbitone (40 mg kg^{-1});

Groups 18-20: AcMT8I at doses of 5.6, 7.5 and 11.25 mg kg^{-1} respectively dissolved in propylene glycol);
Groups 21-29: AcMT81 at doses of 5.6, 7.5 and 11.25 mg kg^{-1} respectively injected one to each group 30 minutes prior to the administration of either diazepam (3 mg kg^{-1}) or chlorpromazine (10 mg kg^{-1}) or pentobarbitone (40 mg kg^{-1}); Groups 30-32: BzMT8l at doses of 8.7, 12.42 and 21.75 mg kg^{-1} respectively (dissolved in propylene glycol);
Groups 33-41: BzMT8I at doses of 8.7, 12.42 and 21.75 mg kg^{-1} respectively injected one to each group 30 minutes prior to the administration of either diazepam (3 mg kg^{-1}) or chlorpromazine (10 mg kg^{-1}) or pentobarbitone (40 mg kg^{-1}).
For narcotic analgesics the design of the experiment was as follows:
Group 1: Vehicle control (5 ml kg^{-1}, propylene glycol);
Group 2: Normal saline (0.9%, w/v; 5 ml kg^{-1});
Group 3: Morphine hydrochloride (5 mg kg^{-1});
Groups 4-5: MT8I at doses of 4.5, 6.0 and 9.0 mg kg^{-1} respectively (dissolved in propylene glycol);
Groups 7-9: MT8l at doses of 4.5, 6.0 and 9 mg kg^{-1} respectively injected one to each group 30 minutes prior to the administration of morphine (5 mg kg^{-1}).
Groups 10-12:AcMT8I at doses of 5.6, 7.5 and 11.25 mg kg^{-1}respectively (dissolved in propylene glycol);
Groups 13-15: AcMT81 at doses of 5.6, 7.5 and 11.25 mg kg^{-1} respectively injected one to each group 30 minutes prior to the administration of morphine (5 mg kg^{-1});
Groups16-18: BzMT81 at doses of 8.7, 12.42 and 21.75 mg kg^{-1} respectively (dissolved in propylene glycol);
Groups 19-21: BzMT81 at doses of 8.7, 12.42 and 21.75 mg kg^{-1} respectively injected one to each group 30 minutes prior to the administration of morphine (5 mg kg^{-1}).

Statistical analysis

The data were expressed as Mean ± SEM. The unpaired student's t- test was applied to evaluate the statistical significance of the data obtained, considering $p<0.05$ as a limit of significance.

Results:

Sedative action

The sleep induced by diazepam, chlorpromazine and pentobarbitone was prolonged following the administration of MT81, AcMT81 and BzMT81. The prolongation of sleeping time caused by the test compounds (dissolved in propylene glycol) is greater than that of PG (vehicle control group) alone ($p<0.01$) and this effect is dose-dependent. The results are summarized in Table-1.

Table – 1

The effects of PG, MT81, AcMT81 and BzMT81 on pentobarbitone, diazepam and chloropromazine induced sleeping time in mice.

Average sleeping time in minute (Mean ± SEM)

Drugs	Response of the test compound alone	With diazepam (3 mg/kg body weight)	With chlorpromazine (10 mg/kg body weight)	With pentobarbitone (40 mg/kg body weight)
Saline	-	66.3±0.9	55.6±1.40	34.5±0.7
Propylene glycol (5 ml kg^{-1})	-	69.0±4.9	73. ±32.37***	41.9±0.33***
MT81:				
(4.5 mg/kg^{-1})	-	78.6±2.36***	291.4±3.19***	81.75±3.55***
(6.0 mg/kg^{-1})	-	157.5±5.95***	312.2±9.65***	116.4±11.84***
(9.0 mg/kg^{-1})	103.4±18.52	212.67±8.91***	All sleep till 7 hrs Dead after 24 hours	2.4.8±8.49***
AcMT81:				
(5.6 mg/kg^{-1})	-	135.8±4.43***	74.4±8.92*	73.25±7.77***
(7.5 mg/kg^{-1})	-	214.8±9.51***	80.2±2.85***	78.6±3.74***
(11.25 mg/kg^{-1})	-	Deep sleep till 6 hours, Death after 24 hours	128.4±16.84***	117±8.82***
BzMT81:				
(8.7 mg/kg^{-1})	-	377.4±5.4***	216.5±8.89***	119.8±7.39***
(12.42 mg/kg^{-1})	-	Deep sleep till 6 hours	230.0 ± 23.16***	157.25±4.17***
(21.75 mg/kg^{-1})	-	All dead after 24 hours	Deep sleep till 6 hrs. All dead after 24 hrs.	301.6±13.87***

No. of animals per group = 10. *P<0.05, **P<0.01, ***P<0.001

Effect on hypothermia

The figure 1, 2 & 3 indicates that the rectal temperature began to fall after administration of PG as well as MT81, AcMT8l and BzMT81. But the fall of temperature after the treatment of the test substances is markedly greater in each case than that of PG alone.

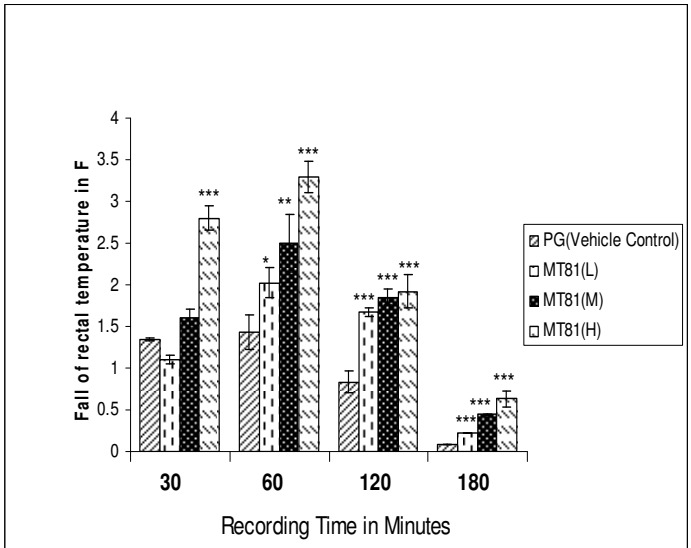


FIG 1: HYPOTHERMIA INDUCED BY MT81 IN MICE

No. of animals per group= 10.*P<0.05, **P<0.01, ***P<0.001

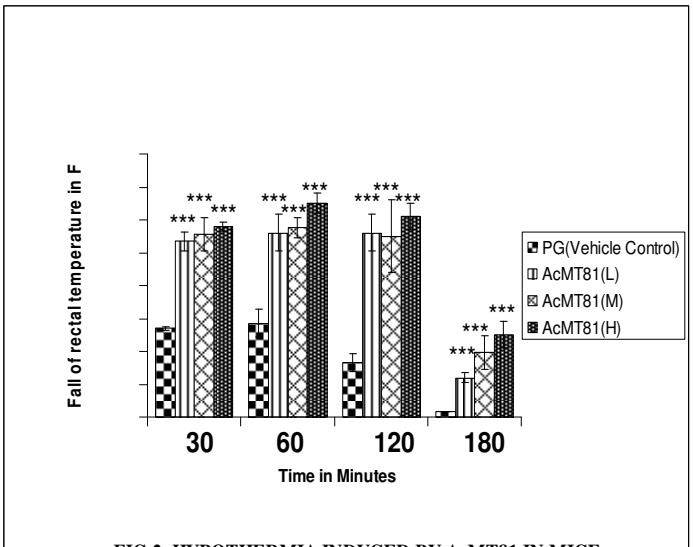


FIG 2: HYPOTHERMIA INDUCED BY AcMT81 IN MICE

No. of animals per group= 10. *P<0.05, **P<0.01, ***P<0.001

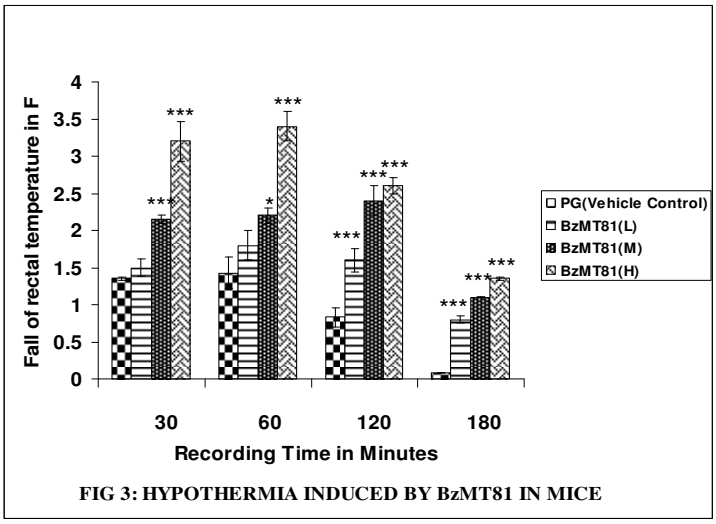
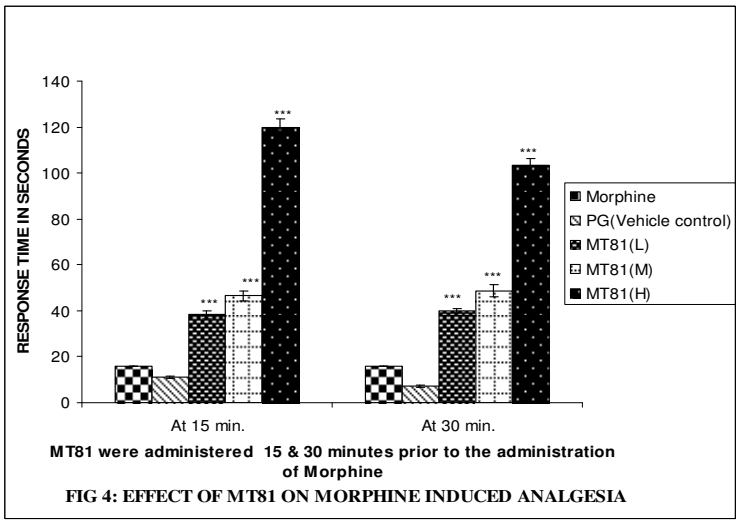


FIG 3: HYPOTHERMIA INDUCED BY BzMT81 IN MICE

No. of animals per group= 10. *P<0.05, **P<0.01, ***P<0.001

Effect on morphine- induced analgesia

MT81, AcMT81 and BzMT81 (each at three dose levels) potentiate the analgesic action of morphine significantly (P≤0.001) in a dose - dependent manner (Fig 4, 5 & 6).



MT81 were administered 15 & 30 minutes prior to the administration of Morphine

FIG 4: EFFECT OF MT81 ON MORPHINE INDUCED ANALGESIA

No. of animals per group= 10. *P<0.05, **P<0.01, ***P<0.001

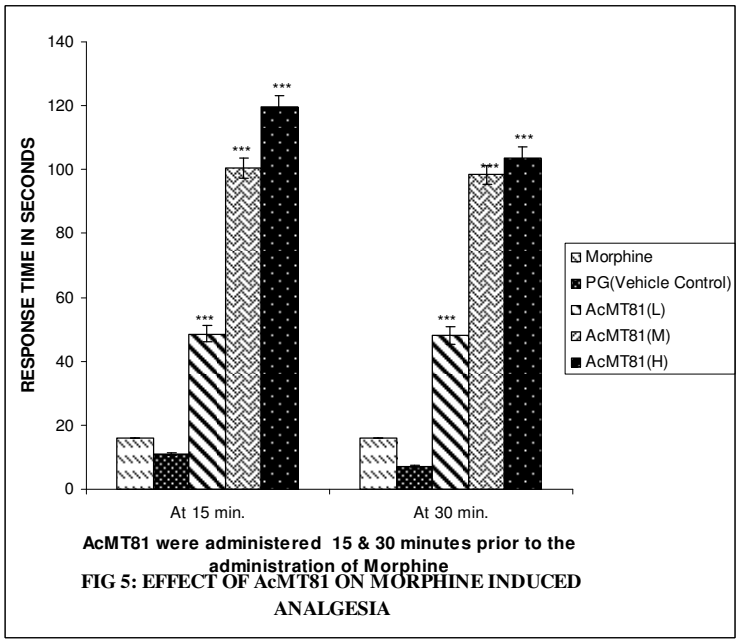


FIG 5: EFFECT OF AcMT81 ON MORPHINE INDUCED ANALGESIA

No. of animals per group= 10. *P<0.05, **P<0.01, ***P<0.001

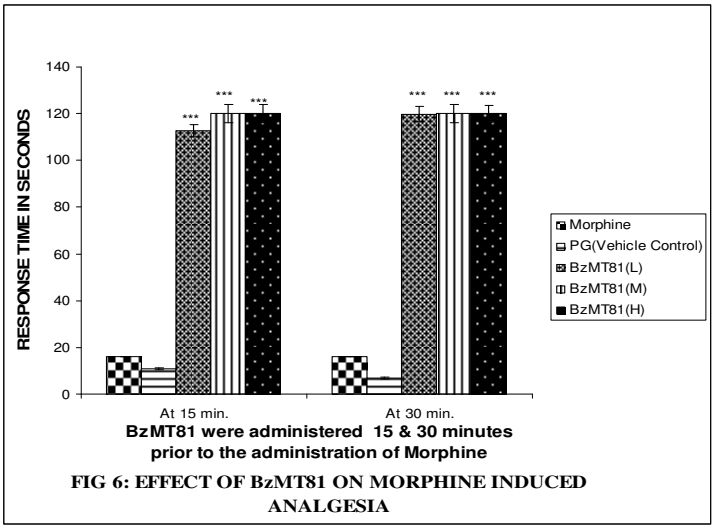


FIG 6: EFFECT OF BzMT81 ON MORPHINE INDUCED ANALGESIA

No. of animals per group= 10. *P<0.05, **P<0.01, ***P<0.001

Discussion

The prolongation of sleeping time caused by the test compounds (dissolved in PG) is greater than that of PG alone ($P < 0.01$) and this effect is dose-dependent. There are some compounds, which prolong the sleeping time induced by certain sedative and hypnotic compounds by vascular mechanism affecting the absorption of the drug from the site of injection, its penetration into the blood-brain barrier or its breakdown or excretion. The test substances may enhance the sleeping time by any of the above-mentioned effects. Many investigators (25) have reported that a decrease in oxygen consumption increases the prolongation of pentobarbitone hypnosis. There are also reports regarding the possible inhibition of mitochondrial respiration by mycotoxins. MT81, AcMT8I and BzMT81 may decrease the mitochondrial respiration. Numerous reports indicate most of the compounds to have hypnotic prolonging activity (26) can lower the body temperature to a noteworthy extent.

From the figures 1, 2, and 3, it is also apparent that the hypothermia produced by MT81 and its structural analogues occurs in a dose-dependent manner. Drugs that produce hypothermia directly or indirectly stimulate dopamine receptors and increase dopaminergic activity (26). Moreover, drugs that potentiate the effects of sedatives produce hypothermia due to decreased level of acetylcholine or increased level of 5-hydroxytryptamine. Thus we may suggest that sedation and hypothermia produced by the test compounds may be due to either increased dopaminergic activity or increased 5-HT or inhibition of calcium permeability as these compounds produce muscle relaxant action, too.

It was reported that 9, 10-anthracene derivatives are psychotropic drugs. As MT81, AcMT8I and BzMT81 are showing a considerable sedative effect and as all these compounds are anthraquinone derivatives, it is expected that the sedative effect be due to the anthraquinone moiety.

Experimentally it is evident that MT81, AcMT81 and BzMT81 (each at three dose levels) like other drugs (27) potentiate the analgesic action of morphine significantly ($P \leq 0.001$) in a dose - dependent manner (Fig 3, 4 & 5). As the three compounds tested produced hypothermia in mice, their analgesic effect may be mediated by inhibition of post- synaptic specific sensitive mechanism, which demonstrates central antinociceptive effects of acetylsalicylic acid and paracetamol. It has also been reported (28) that morphine induced analgesia is associated with the reduction in norepinephrine activity in the nervous system particularly at α-adrenergic receptor sites either by depleting endogenous levels via dopamine β-hydroxylase inhibition or blocking the effects at the receptor level. MT8I, BzMT81 and AcMT8I have showed CNS depressant action and have reduced the norepinephrine content in mice. This can be related to the analgesic property of the test compounds.

Thus based on experimental evidences so far, it is suggested that the compounds act centrally as well as peripherally. Further investigations along

this line will probably pinpoint the exact mechanism and pathway of analgesic activity (29), sedative effect and hypothermic action of MT81, AcMT81 and BzMT81.

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