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ACUTE TOXICOLOGY AND NEUROBEHAVIORAL STUDIES ON A NEW 7-AZAINDOLE DERIVATIVE

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ABSTRACT

In view of the biological significance of 7-Azaindole containing compounds, we report the synthesis and the evaluation of 1-methhyl -4-{1H-pyrrol [2, 3-b] pyridine-3-yl-1, 2, 3, 6-tetrahydropyridine (A_1) for acute toxicological effects and neurobehavioral studies using animal module. The compound 1-methhyl -4-{1H-pyrrol [2, 3-b] pyridine-3-yl-1, 2, 3, 6-tetrahydropyridine (A_1) was synthesized in laboratory and subjected to acute toxicological observations. The compound was investigated with emphasis to determine the LD_{50} and to observe neurobehavioral effects in rats and mice. Six dose levels, i.e., 10, 25, 50, 75,100 and 125 mg/kg for rats was selected for the animal model. The LD_{50} was determined by Arthmatic method of Karber. Prominent signs and symptoms of toxicity was observed. Data plotted using functional observation battery method indicated increase in severity with the increase of dose. The study revealed that 1-methhyl -4-{1H-pyrrol [2, 3-b] pyridine-3-yl-1, 2, 3, 6-tetrahydropyridine (A_1), a 7-Azaindole compound as neurotoxic.

KEY WORDS: 7.Azaindole, LD_{50}, Neurobehavioral studies, Arthmeic method of Karber, Functional Observational Battery.

1. INTRODUCTION

Biological activity of indole derivatives have been studied and reported on a wide scale over the past decades which include anti-inflammatory activity[1] , Analgesic activity[2] , Antimicrobial activity[3] , anticonvulsant activity[4] , Antitumor activity [5] , antifungal activity [6] , antiviral activity .[7] However, not much attention is diverted by the researchers to evaluate the toxicity of indole derivates which is quite surprising. Therefore, present study was taken into consideration to evaluate toxicity of a 7-Azinadole.

Among the various types of toxicological studies, acute toxicity studies provide information about the overall profile of a drug's activity, magnitude of its toxicity, and overall effects. [8] One of the basic step in toxicological assessment of a new substance is the determination of its toxicity after a single exposure of that substance. This provides the fundamental clues regarding the type of toxic effects which the substance may possess and also gives information about the lethal dose. The most frequently used acute toxicity test includes determination of the median lethal dose (LD_{50}) of the compound. The LD_{50} has been defined as "statistically derived expression of a single dose of a material that can be expected to kill 50% of the animals".[9]

Essentially all chemicals of biological interest undergo acute toxicity testing. The purpose of the test is to determine the order of lethality of the substance. Careful evaluation of the symptoms, produced by administrating a bioactive chemical, can provide significant information to help in characterizing the toxicant's mode of action, i.e., neurological, cardiovascular, respiratory, or others.[10] The use of behavioral procedures in toxicological assessments has increased in recent years. [11] More precisely it can be defined as the dose-effect relationship between drugs that is producing the effects with the effects that are produced as a result of exposure of that drug. [12] The usual first phase of acute toxicity testing examines the dose-response relationships based on systematic observations of responses, which are normally included in functional observation batteries, generally use to judge simple and innate behaviors.[13] In FOB (Functional Observational Battery), sometimes the presence and in some cases the severity, of behavioral and neurological signs are rated.[14] Both the range of doses and their effects are considerable, so that scientists may not have to perform other more specific or sensitive tests on animals at the higher doses.[15]

2. MATERIALS AND METHODS

2.1. General experimental procedures and chemicals used

Reagents were purchased from Aldrich Chemical Company. All solvents were analytical grade. Reactions were monitored by TLC using pre-coated silica gel, GF-254 and Analytical thin layer chromatography (TLC) was performed on silica gel (Kieselgel 60, 254, E. Merck) precoated 0.25 mm plates. Visualization was accomplished with ultraviolet light (UV) at 254 and 365 nm UVP UVLS-26 Series (Cambridge). Ultraviolet (UV) spectra was recorded in methanol on a Hitachi U-3200 spectrophotometer. Infra-Red (IR) spectra was measured on a Shimadzu IR 460 spectrophotometer using KBR disc. Mass spectra (MS) was determined on Varian Massen spectrometer MAT 311A spectrometer. Nuclear magnetic resonance (1HNMR) spectra was recorded in methanol on AVANCE AV 300 spectrometer operating at 300MHz.

2.2.Synthesis of 1-methhyl -4-{1H-pyrrol [2, 3-b] pyridine-3-yl-1, 2, 3, 6-tetrahydropyridine (A_1)

Equimolar quantity of 7-azaindole and N-metyl -4- piperidone was dissolved in ethanol separately Two pallets of sodium hydroxide were then added to a solution of 7-azaindole and were allowed to dissolve too. Both the solutions were mixed and stirrd at about 60^{o}C. the thick yellow colour precipitates were appeared after half an hour. Filter and washed it with water to remove the sodium hydroxide. Recrystallization was carried out using hot ethanol to get fine leafy crystals of 1-methhyl -4-{1H-pyrrol [2, 3-b] pyridine-3-yl-1, 2, 3, 6-tetrahydropyridine (A) were obtained.

2.3. Acute toxicology and neurobehavioral studies

For the determination of acute toxicity male and female Sprague-Dawley rats weighed around 200grams were used. There were 7 groups of 6 animals, each group labeled with numeric numbers 1 to 6. The compound was administered intraperitonially in 6 gradients of 10, 25.50, 75,100, and 125 mg/kg for rats. The control groups (group 1) received 0.2ml of DMSO, under similar experimental conditions and through the same route. The animals were weighed before the administration of the drug.

2.5. Determination of LD_{50}

The estimation of lethal dose that produces 50% death in animals was done by method described below:

2.5.1.Arithmetic method of Karber

The interval mean of the number dead in each group of animals was used as well as the difference between doses for the same interval. The product of interval mean and dose difference was obtained. The sum of the product was divided by the number of animals in group and the resulting quotient was subtracted from the dose that causes 100% motility in order to obtain LD_{50} value.

$$LD_{50} = LD_{100} - \sum \frac{Dd \times Md}{N}$$

Where, LD_{50} = Dose that caused 50% mortality

LD_{100} = Dose that caused 100% mortality

N = No. of animals per group

Dd = Dose difference

Md = Mean death

Dd × Md = Dose difference multiply by the mean death .[16]

2.7. Functional Obsevational Battery

A pilot study was conducted at dose 100mg and the animal was observed carefully for the toxic effect. As the animals showed a detectable effect within 7 hours and after which the animals just showed the same type of effects if not dead. Almost all the animals died within 24 hrs the time slot of the study was divided into 15 min, 7 hrs and 24hrs. The animals were observed uninterrupted for one hour in an open field to mark the effects. The study was carefully designed to collect data for both LD_{50} and behavioral study in order to sacrifice fewer animals. For this purpose animal was observed at 100 mg dose and then the dose was decreased gradually to a dose with minimal effects. A higher dose of more than 100mg was used for rats to check the more toxic effects. The test variables were selected in order to have a quick check weather the chemical has neurotoxic effects or not and at which dose level and this type is called first-tier testing. [17]

The neurotoxicity of a compound is important to fulfil regulatory issues of many compounds, for this purpose changes in behavior is among routine measurements. There are mainly two recommended levels of neurobehavioral testing in which primary level, includes functional observational battery and motor activity. [18] There are numerous measures in FOB but in this study the protocol of FOB has been adopted and modified according to the need and availability of lab equipments. The FOB consisted of home cage, handling and open field. The FOB was consisted of 13 variables in order to assess the acute toxicity of the

compound following five domains of behavioral responses, these were behavioral changes, CNS depression, CNS excitation, dermal observation and autonomic effects. The rats were scored for changes in behavior at 5 selected doses for behavioral change , motor activity, ataxia, tremors, tonic convulsions, respiratory rate, cyanosis, piloerection, urination , defecation, staub tail and abdused hind limbs (Table 2). The effects were recorded after 15 min, 7Hrs and 24hrs in a simple manner of positive and negative signs if there is any effect or no effect respectively while double positive sing is used for greater effect .

The FOB protocol in this study was based on the procedural details and scoring criteria was self done. A key is also provided beneath the table.[19] The FOB was conducted in the morning and the animals were constantly monitored for 15 min and then every hour for 7 hrs and lastly after 24 hrs. The observer used in the study was self trained and was the only one measuring the behavioral changes. Here it should be clear that the individual measuring the behavioral changes is not a part of daily care of the rats. The FOB was conducted in the morning in a separate room , limited people were allowed to enter the room the rats were removed from their respective cages and placed in an open field for continuous 1 hour during which the animal were observed constantly for the changes in behavior. For aggressive and fearfulness animals were handled and shifted to different places of the open field and the change was recorded. For autonomic effects like urination the urine pool in and out of open field is observed, while in defecation quantity of fecal mass was seen. Motor activity and ataxia also observed in open field.

3.RESULTS AND DISCUSSION

3.1 Analytical and spectral data of the derivative

Chemical shifts are reported in ppm. Selected data are reported as follow: chemical shift, multiplicity (s = singlet, m = multiplet), coupling constant J (Hz), number of proton (1H = one proton, 2H = two protons, …….nH = n proton

The identification of thre derivative, figure 1 was achieved using different spectroscopic techniques. Data given below.

^{1}HNMR (MeOD, 300 MHz) δ: 9.55 (s, 1H, NH-10), 8.29-8.15 (m,1H, H-13), 7.26-7.06 (m, 3H, H-14, H-15,H-9),6.15-6.13(m,1H, H-5), 3.16-3.13(m,1H,H-6), 2.70-2.14(m, 2H, H-2,H-3), 1.68 (S,1H,H-7).

EIMS m/z: 213 (M^+, $C_{13}H_{15}N_3$), 198, 184, 142,131,115, 94.

HR-EIMS: 213.2772 ($M^+C_{13}H_{15}N_3$) Calculated 213.2784.

IR ν_{max} (KBr) cm^{-1}: 1571.3Hz

UV λ_{max} (MeOH) nm: 202, 235, 251, 289.

State: yellow colour crystals, yield: 90%

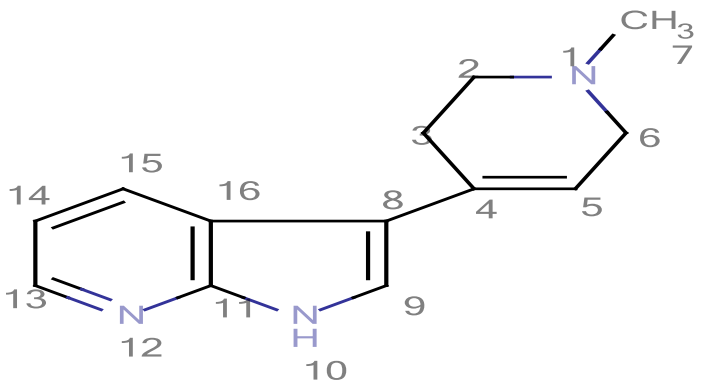

Figure (1) 7-Azaidole derivative 1-methhyl -4-{1H-pyrrol [2, 3-b] pyridine-3-yl-1, 2, 3, 6-tetrahydropyridine (A_1)

Table 1: Arithmetic method of Karber for determination of LD50 in Rats

GROUP	DOSE mg/kg	DOSE DIFFERENCE (Dd)	NO OF DEAD	NO OF ANIMAL Per GROUP	MEAN DEAD (Md)	PROBIT (DdxMd)
1	10	0	0	6	.5	0
2	25	15	1	6	1.5	22.5
3	50	25	2	6	3	75
4	75	25	4	6	4.5	112.5
5	100	25	5	6	5.5	137.5
6	125	25	6	6	6	150
						∑ 497.5

$$LD_{50} = LD_{100} - \sum \frac{Dd \times Md}{N}$$

$$LD_{50} = 125 - \frac{497.5}{6}$$

$$= 125 - 82.91$$

$$LD_{50} = 42.09 \text{ mg/kg}$$

Table 2 : Showing the FOB and their outcomes in rats

Domain	10mg			25mg			50mg			75mg			100mg			125mg		
specific tests	15min	7H	24H	15min	7H	24H	15min	7H	24H	15min	7H	24H	15min	7H	24H	15min	7H	24H
Behavior																		
aggressive	_	+	_	_	+	_	+	_	_	++	+	_	++	++	_	+	++	
fearfull	+	+	_	+	+	+	++	+	+	++	+	+	++	+	+	++	++	
CNS depression																		
motor activity	_	_	_	+	+	_	+	_	_	++	+	_	++	++	_	++	++	
ataxia	_	_	_	+	+	_	+	_	_	++	+	_	++	++	+	+	++	
CNS Excitation																		
Tremors	_	_	_	_	+	_	++	_	_	++	+	_	++	+	_	++	++	
Tonic convulsions	_	+	_	++	_	_	++	_	_	+	+	_	++	+	_	++	++	
Respiratory Rate	_	+	+	+	+	_	++	+	_	++	+	_	++	+	_	++	++	
Dermal Observation																		
Cynosis	_	_	_	_	+	_	++	+	_	++	+	_	++	++	+	++	++	
Piloerection	_	+	_	+	+	_	++	+	_	++	+	_	++	+	_	++	++	
urination	+	+	_	+	+	_	+	+	_	++	+	_	++	+	_	++	++	
defecation	+	+	_	+	+	_	+	+	_	++	+	_	++	+	_	++	++	
staub tail	_	_	_	+	+	_	++	_	_	++	+	_	++	+	_	++	++	
abdused hind limb	_	_	_	+	_	_	+	+	_	++	+	_	++	+	_	++	++	

Where time (T1,T2 & T3) are 15min,7hrs and 24hrs respectively, where 0 = not done , - = no effect, + = mild effect, + + = strong effect, ↑ = mild stimulation, ↑↑ = Strong stimulation, ↑ = mild depression, ↑↑ = strong depression

DISCUSSION

The review of literature organized during present studies indicated a significant gap in terms of toxicological studies of the 7-Azaidole derivative. More emphasis have been given by the reserachers to evaluate their biological significance to explore the possibility of their introduction as a therapeutic agent. In view of this, present study was taken into consideration. It is believed that the major hindrance in understanding the true potential of 7-Azaidole compound is the lack of preliminary data in support of the effect it carries after a single exposure. It is therefore pertinent to establish a single dose toxicological assessment which will give the basic information regarding the minimum lethal dose through the determination of LD_{50} while behavioral observational method provides general screening of the potential pharmacological action a drug may carry.

Acute toxicological studies of compound 1-methhyl -4-{1H-pyrrol [2, 3-b] pyridine-3-yl-1, 2, 3, 6-tetrahydropyridine were carried out to find out the minimum lethal dose and the possible effects it may carry using rats as animal model. The calculation of minimum lethal dose or LD_{50} was carried out by Arithmetic method of Karber (Table 1) while the

behavioral toxicological study was carried out by using a screening battery known as Functional Observational Battery or FOB (Table 2). The observations made to determine both the tasks were carried out at the same time using the same sets of animals. The LD_{50} with the Karber method came out to be approximately 42 mg/kg in rats (Table 1).

The neurobehavioral effects of rats were recorded in Table 2 . The effects were recorded from just after the time of exposure of medicine with intervals ranging from 15min, 7 hrs and 24 hrs respectively. The neurobehavioral effects of the compound were fairly characteristic of other compounds belonging to the same class of alkaloid that is indoles. It was reported that behavioral changes in rats after exposure of an indole derivative compound, the animals showed increased in the magnitude of effects with increased dose level. [20]

Table 2 shows the change in behavior in rats, the animals shows the augmentation in aggressiveness and fearfulness with the increase in dose levels. Among the CNS depressing activities two effects were seen very clearly and was also plotted in order of increasing intensity in the same table while the CNS excitation was also recorded. No considerable changes in behavioral effects were seen at lower doses but slight increased in intensity with increasing dose was observed .An increase in urination and defecation was observed, with the exception that slightly depressed motor activity and ataxia was observed just after 15 minutes of the administration of drug. piloerection staub tail and abdused hind limbs were also recorded. In rats the abduced hind limbs were seen just after 15 minutes of administration of drug but it started working again after three hours. The grip strength was also slightly decreased in animals but the effects faded within 24 hours. At a little higher dose that is 75 mg/kg the dermal effects increased, Cyanosis was seen at this dose level in almost all the animals.

At 100 mg / kg dose the effects intensified and almost all the animals died within 24 hours except for few servivors. At dose 125 mg all the rats died within 24 hours of administration of drug. Decreased motor activity was also observed in animals at all levels of dosage. Except that the magnitude of the effect was directly proportional to the level of dose administered , that is increased with higher dose. [21] Cyanosis was seen at higher doses while autonomic effects like piloerection was seen at higher doses and increased urination and defecation was observed at all dose levels. The common clinical signs that have been observed during the toxicological assessment gives us a clue regarding the organ, tissue or system which is most likely involved in giving those responses.[22] For instance Cyanosis may be caused due to

pulmonary or cardiac insufficiency; pulmonary edema may also be the cause. While decreased or increased in spontaneous motor activity shows connection with somatomotor, and central nervous system. Ataxia can be caused through the involvement of the central nervous system, sensory or autonomic nervous system. In tremors and convulsions both neuromuscular and central nervous system may be involved. Piloerection changes in frequency of defecation and urination can be a cause of autonomic nervous system.

The present study has given a comprehensive data to support in context of acute toxicology. These effects can be characterized as slightly reversible at low doses that is at doses 10, 25, 50 while at higher doses the effects seen were irreversible. Behavioral evaluations are considered a key component in neurotoxicity testing. In the acute studies reported here behavioral change was observed in almost all the doses. Tremors were also observed in all animals which increased with increasing dose level, the tremoginic property of some indoles were also reported. [23] Convulsions were also reported for some animals at higher doses and all animals dosed at 100 and 125 mg had convulsions and died acutely. Few animals in lower doses which had convulsions died subsequently. Other stimulatory effect which has been reported after intraperitonial administration of the tested compound increased respiratory rate. a study on antidepressant activity of some indole containing compounds can explain this effect.[24]

CONCLUSION

The 7-Azaidole derivative, 1-methhyl -4-{1H-pyrrol [2, 3-b] pyridine-3-yl-1, 2, 3, 6-tetrahydropyridine observed to be both central and autonomic acting drug with effects on GI motility. After retaining the symptoms as mentioned in discussion it can be assumed that the compound might possess neurotoxic effects but the mechanism of action is not clear. So it is suggested that an extended and more elaborated studies should be organized for the evaluation of mode of action and neurotoxic effects.

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