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NEURO-TOXICOLOGICAL IMPACTS OF DATURA METEL LINN. (FAMILY: SOLANACEAE) LEAVES EXTRACT IN MICE

FAMİLYASI PATLICANGİLLER OLAN BORU ÇİÇEĞİ(DATURA METEL LINN.)'NİN EKSTRESİ FARELERDE NÖRO-TOKSİK ETKİLER BIRAKIR

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Abstract

Datura metel leaves and seeds are widely used in ethno-therapeutic management of Rheumatic pain, asthma and insomnia. Its use has been associated with adverse alteration in behavior which has triggered interest in its safety on the central nervous system. This study was therefore designed to evaluate acute neuro-toxicological effects of aqueous –methanol extracts of Datura metel in mice. Oral Acute toxicity studies of the leaf extract was carried out in mice. The effects of the extract (25-100 mg/kg body weight orally) on total locomotive activity, motor coordination and spatial memory in Y-maze were evaluated in mice. The effect of Datura metel extract (25-100 mg/kg) in the presence of either atropine (0.3 mg/kg b.w.) or naloxone (0.5 mg/kg b.w., i.p.) on total locomotive activity in an open field apparatus was carried out with the view of predicting its mechanism of action. The oral median lethal dose (LD50) was greater than 2000 mg/kg b.w. The extract produced significant decrease ($p<0.05$) in total locomotive activity of the treated mice in the open field apparatus. The extract significantly ($p<0.05$) shortened the time spent on the rota-rod by mice treated with the extract and reduced spontaneous alternation behavior. Datural metel leaves extract produced in mice neuro-toxicological effects characterized by sedation and hypokinesia, motor coordination impairment and disruption of short term memory.

Keywords: Datural metel, extract, mice, neurotoxicity, amnesia.

Özet

Boru çiçeği yaprak ve tohumları romatizma ağrısı, astım ve uykusuzluğun etno-terapötik uygulamasında yaygın biçimde kullanılmaktadır. Kullanımı, davranışlardaki olumsuz değişimlerle alakalı olması ve merkezi sinir sistemi üzerindeki güvenirliliği bu çalışmayı tetikledi. Bu çalışma Boru Çiçeğinin sulu metanol ekstresinin farelerdeki akut nöro-toksik etkilerini değerlendirmek için tasarlandı. Yaprak ekstresinin oral akut toksisite çalışması fareler üzerinde gerçekleştirildi. Ekstrenin Y-labirentteki toplam lokomotif aktivite, motor koordinasyon ve uzamsal hafıza üzerindeki etkileri (ağız yoluyla 25mg-100 kg vücut ağırlığı) farelerde değerlendirildi. Boru Çiçeği ekstresinin (25-100 mg/kg) açık alan aparatındaki toplam lokomotif aktivite üzerindeki etkisi nalokson (0.5 mg/kg b.w., i.p.) ya da atropine (0.3 mg/kg vücut ağırlığı)' varlığında gerçekleştirilip hareket mekanizmasının tahmini maksadıyla gözlemlendi. Oral medyan letal dozu (LD50) 2000 mg/kg vücut ağırlığından daha fazlaydı. Ekstre, açık alan aparatında muamele edilen farelerin toplam lokomotif aktivitesinde önemli bir azalış ($p<0.05$) meydana getirdi. Bu ekstre, işleme alınan farelerin rota-rod üzerinde harcadıkları zamanı önemli derecede ($p<0.05$) kısalttı ve eşzamanlı değişim gösteren davranışları da azalttı. Boru Çiçeği yaprak ekstresi, farelerde sakinlik, uyuşukluk, motor koordinasyon bozukluğu ve kısa süreli hafıza bozulmasıyla nitelenen nöro-toksik etkiler meydana getirmiştir.

Anahtar Kelimeler: *Boru Çiçeği, ekstre, fareler, nörotoksisite, amnezi*

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1. Introduction

The medicinal plant Datura metel (DM) (Fam. Solanaceae) has been used in ethno-therapeutic management of asthma, insomnia and rheumatic pain. The smoke from the burning leaf is inhaled for the relief of asthma and bronchitis. The fruit juice is applied to the scalp for the treatment of falling hair and dandruff. Seeds and leaves of D. metel were reportedly used to sedate hysterical and psychotic patients (Gary et al., 2005). The decoction of D. metel leaf has been reported to be effective in management of madness, epilepsy and depression. It has been used as a narcotic and local anesthetic drug in many societies (Das et al., 2012). The bitter narcotic plant relieves pain and encourages the healing process. The seeds of the plant are medicinally the most active. Externally, the plant is used as a poultice in treating fistulas, abscesses wounds and severe neuralgia (Shagal et al., 2012).

Phytochemical studies of the plant revealed the presence of Scopolamine in the plant, which makes it a potent cholinergic-blocking hallucinogen that has been used to calm schizoid patients. Its leaves, containing hyoscyamine

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and atropine, can be used as an immensely powerful mind-altering drug (Strahil et al., 2006). There are reports of intoxication produced by the plant in teenagers who used the plants leaves for non-medical purposes (Ertekin et al., 2005). Hallucinogenic plants have been use as mind altering agents since the beginning of the recorded history. Apart from the therapeutic potential inherent in this plant, it enjoys patronage from criminals who seeks for mood alteration effects (sensory experience of something that does not exits out -side mind).

There are unsubstantiated claims of its leaves being used as hallucinogen by teenagers in parts of Nigeria. This study was therefore designed to study the effects of Datura metel leaves extract on spontaneous motor activity, spatial memory, motor co-ordination and cholinesterase activity in mice with the view to provide scientific evidence for its acclaimed neurotoxicological effects.

2. Materials and methods

2.1. Drugs and reagents

Methanol (Sigma- Aldrich, UK), Atropine (Sigma Aldrich, UK), Naloxone (Sigma Aldrich, UK) were used for the study.

2.2. Plant material

Fresh leaves of Datura metel was collected from Jiwa, Abuja, Nigeria. It was identified by Mallam Ibrahim Muazzaman ethnobotanist and authenticated by Mrs. Ugbabe Grace a taxonomist with the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. Herbarium specimen (NIPRD/H/6455) was prepared and deposited for future references.

2.3. Preparation of plant extract

The leaves were cleaned, air-dried and crushed into fine powder using a pestle and mortar. Fifty grams of the powdered leaves was cold macerated with 0.5 L of 70% v/v methanol in water for 72 h at ambient temperature (28 – 30 oC). The resultant mixture was filtered using Whatman filter paper (No.1) and the filtrate concentrated to dryness in vacuo at 40 oC using Rotary Evaporator to give 9 g (18 % w/w) of a dark green hygroscopic powder The dried extract was stored in a dessicator. .

2.4. High Performance Liquid Chromatography Analysis

High performance liquid chromatography analysis was performed on the methanol extract of using (Bienvenu et al., 2002) method with some modifications. The chromatographic system includes Shimadzu HPLC system consisting of Ultra-Fast LC-20AB equipped with SIL-20AC auto-sampler; DGU-20A3 degasser; SPD-M20A UV-diode array detector; column oven CTO-20AC, system controller CBM-20Alite and Windows LC solution software (Shimadzu Corporation, Kyoto Japan); column, VP-ODS 5µm and dimensions (150 x 4.6 mm). The chromatographic conditions included mobile phase: solvent A: 0.2% v/v formic acid; solvent B: acetonitrile; mode: isocratic; flow rate 0.6 ml/min; injection volume 10 µl of 250 mg/ml solution of extract in methanol; detection UV 254 nm. The HPLC operating conditions were programmed to give mobile phase comprising solvent B 15% and solvent A 85 %. Column oven temperature was 40 0C. Atropine and scopolamine reference standards were analysed under the same HPLC conditions to establish their retention times. The total run time was 15 minutes.

2.5. Animals

Adult male Swiss albino mice (22–25 g) obtained from Animal Facility Centre (AFC) of National Institute for Pharmaceutical Research and Development (NIPRD) were used for this study. The mice were housed in transparent plastic cages padded with wood shavings, under standard conditions of temperature, relative humidity and light/dark cycles (12/12 h). They were fed with standard rodent chow and water ad libitum. Mice were handled and used for study accordance with the revised 1996 National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80 23).

2.6. Drugs and dosages

The following drugs and dosages were used for pharmacological screening: Normal saline (10 ml/kg body weight) for control group, Naloxone (0.5 mg/kg body weight) dissolved in normal saline; aqueous methanol extract of Datura metel leaves (25, 50 and 100 mg/kg body weight. The extract and normal saline were given by gavage while naloxone was injected intraperitoneally.

2.7. Acute toxicity study

Acute toxicity study was carried out according to the Organization for Economic Co-Operation and Development (OECD) Guidelines No. 423. Three animals were used for each step. The starting dose was selected from one of four fixed levels, i.e., 5, 50, 300, and 2000 mg/kg (b.w. p.o.) In accordance to the OECD recommendations, the starting dose level should be that which is most likely to produce mortality in some of the dosed animals; and when there is no information available on a substance to be tested in this regard; for animal welfare reasons, recommended starting dose is 300 mg/kg body weight. Thus 300 mg/kg body weight was used as the starting dose. The animals were observed for signs of toxicity and pattern of mortality for the first 4 h, then at 24 h and thereafter daily for 14 days.

2.8. Effect on Total locomotive activity

One hour after the administration, mice (N= 6) were transferred to the apparatus which consist of a clear glass box (45 cm×45 cm). The floor was divided by lines drawn into 9 equally sized squares (15 cm x 15 cm). Each mouse was placed individually in the centre of the apparatus and observed for 5 min to record the locomotor (number of

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squares crossed with four paws) (Santosh et al., 2011). After each test, the floor and walls of the apparatus were thoroughly cleaned with 70% ethanol to eliminate possible bias due to odour clue left by previous subject (Lindholm et al., 2012).

2.9. Study design

Group I: Normal saline (10 ml/kg body weight)

Group II: Datura metel methanol leaves extract 25 mg/kg body weight

Group III: Datura metel methanol leaves extract 50 mg/kg body weight

Group IV: Datura metel methanol leaves extract 100 mg/kg body weight

Group V: Datura metel methanol leaves extract 25 mg/kg body weight + Naloxone (0.5 mg/kg body weight)

Group VI: Datura metel methanol leaves extract 100 mg/kg body weight + Naloxone (0.5 mg/kg body weight)

Group VII: Datura metel methanol leaves extract 25 mg/kg body weight + atropine (0.3 mg/kg body weight)

Group VII: Datura metel methanol leaves extract 100 mg/kg body weight + atropine (0.3 mg/kg body weight)

2.10. Effect on Motor function

After administration of extract or normal saline motor performance of mice (n=6) was assessed using rota-rod apparatus which consists of a bar with a diameter of 3.0 cm, subdivided into five compartments by a disk of 24 cm in diameter. The bar rotated at a constant speed of 16 revolutions per min. A preliminary trial was carried out to select mice for the study on the day of experiment, with exclusion criteria being inability to remain on the rota-rod bar for three consecutive periods of 60 s each prior to treatment. The motor coordination function was assessed on the basis of the number of falls from the rota-rod in 180 s.

2.11. Effect on Spatial memory in Y-maze

One hour after administration of normal saline or extract each mouse (n=6) was placed in the Y-maze. The Y-maze is a three-arm horizontal maze (40 cm long and 5 cm wide with walls 10 cm high) in which the three arms are symmetrically separated at 1200. Each mouse was initially placed within one arm (A), and the arm entry sequence (e.g., ABC, CAB, where letters indicate arm codes) and the number of arm entries were recorded manually for 6-minutes. Alternation was determined from successive entries into the three arms on overlapping triplet sets in which three different arms were entered. The percentage alternation for each mouse was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries minus 2), multiplied by 100 as shown by the following equation:

%Alternation = [(Number of alternations) /(Total arm entries-2)] × 100 (Kim et al., 2006; Heo et al., 2009)

2.12. Statistical analysis

Data were analyzed by analysis of variance test followed by Dunnett's test. All the results were expressed as mean ± SEM. $P < 0.05$ was considered significant.

3. Results

3.1. HPLC analysis

HPLC chromatogram of the standardized extract of Datura metel leaf. Seven peaks were detected with retention times of 3.570, 3.976, 4.346, 4.718, 5.226, 6.234 and 6.773 minutes in the HPLC spectrum of Datura metel leaf extract. Scopolamine appeared at retention time of 4.346 minutes and atropine appeared at 6.234 minutes (Fig. 1).

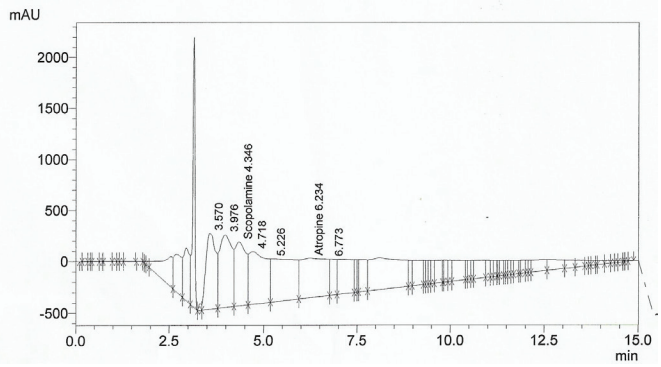

Fig. 1: HPLC chromatogram of the methanol leaf extract of Datura metel in the presence of standard reference atropine and scopolamine.

3.2. Acute toxicity study

Datura metel leaves extract (300 mg /kg body weight p.o.) produced biphasic effect in treated mice. In the first phase it produced restlessness, excitation, and hyper-locomotion while in the second phase mice were observed to be calm, non-responsive to touch, sedated and laboured breathing. Mice treated with the extract at 2000 mg/kg body weight exhibited sedation, laboured breathing and convulsion.

3.3. Effect on Total locomotive activity

The extract (50, 100 and 200 mg/kg body weight p.o.) produced significant ($p<0.05$) decrease in total locomotive activity of the treated mice. The reduced locomotive activity produced by the extract alone was however reversed in the presence of Naloxone (0.5 mg/kg body weight i.p.) as shown. The decrease in speed of mice on open field produced by Datura metel extract was reversed by naloxone (Table 1).

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Table 1: Effect of Datura metel methanol leaves extract on total locomotive activity

Treatment	Total locomotive activity
Normal saline	
10 ml/kg	12.50 ± 0.25
Extract	
25 mg/kg	8.36 ± 0.18*
50 mg/kg	6.85 ± 0.37*
100 mg/kg	4.65 ± 0.48*
Extract (25 mg/kg)+ Naloxone (0.5 mg/kg)	11.75± 0.15
Extract (50 mg/kg)+ Naloxone (0.5 mg/kg)	9.48 ± 0.25
Extract (100 mg/kg)+ Naloxone (0.5 mg/kg)	7.76± 0.25

*Significantly different from the control at p<0.05

3.4. Effect on motor function

The extract significantly (p<0.05) shortened the time spent by treated mice on the rotating rod of the apparatus when compared to the control (Table 2).

Table 2: Effect of Datura metel methanol leaves extract on motor coordination of mice

Treatment	Mean fall off Time from rotarod (Sec)
Normal saline	
10 ml/kg	180.00 ±0.00
Extract	
25 mg/kg	125.52 ± 12.65*
50 mg/kg	56.45 ± 10.49*
100 mg/kg	24.65 ± 0.48*

*Significantly different from the control at p<0.05

3.5. Effect on Spatial memory in Y-maze

The extract (25-100 mg/kg body weight p.o.) produced significant (p<0.05) decrease in the spontaneous alternation behaviour of treated mice in Y-maze task (Table 3).

Table 3: Effect on Spatial memory in Y-maze

Treatment	Mean± SEM Total arm entry	Mean± SEM % Alternation Behaviour
Normal saline		
10 ml/kg	16.56 ±1.65	53.00 ±0.40
Extract		
25 mg/kg	12.32 ± 0.83*	47.00 ±0.56
50 mg/kg	7.32 ± 0.24*	24. 00 ± 0. 15
100 mg/kg	6.36 ± 0.35	18.50 ± 0.75

*Significantly different from the control at p<0.05

4. Discussion

The appearance of Scopolamine at retention time of 4.346 minutes and atropine at 6.234 minutes of the HPLC spectrum of Datura metel leaf extract indicates the presence of these compounds in the extract. This is a useful parameter for identification and classification of substances that may be contaminated or purposely adulterated with Datural metel leaves. It is therefore a potential resource for forensic determination of exposure to Datural metel.

An important step in evaluating CNS drug action is to observe its effect on locomotor activity of the animal. The activity is a measure of the level of excitability of the CNS, and decreased activity results from CNS depression [Ozturk et al., 2002; Tijani et al., 2012). The Datura metel leaves extract significantly decreased the locomotor activity as observed in the results of the open field test. The movement of the treated mice on the open fields was in a disorderly manner when compared to the saline treated group. The ability of naloxone to reverse the depression produced by the extract strongly suggest involvement of opioidergic - receptor in the observed neuro-depression characterized by low activity, freezing on the open field apparatus which accounts for the decreased locomotive activity observed only in the treated animals.

The depressant effect of Datura metel on the Central Nervous System was further supported by the outcome of the rota rod test, which has clearly demonstrated the CNS depressant activity evidenced by decreased fall off time. Rota-rod test is a valid test for predicting motor dysfunction produced by centrally acting drugs to determine possible alterations in the motor coordination ability of the animal, often caused by the use of sedative and antipsychotic drugs. In this test, the difference in the fall off time from the rotating rod between the vehicle and extract treated groups is taken as an index of muscle relaxation. In this study the short fall off time in the extract treated group showed that the extract possesses muscle relaxant effect. This may explain the incoordination in movement observed in all the treated animals on the open field.

The effect of the extract on memory was investigated in the Y-maze apparatus in order to confirm its effect on spatial short term memory. The choice of Y-maze for the study was based on the work of (Kim et al., 2006) which showed that spontaneous alternation behaviour is an indicator of spatial memory. Any agent that increased the spontaneous alternation behavior enhances memory while agents that reduce this parameter produces memory deficit. The extract produced memory deficit as evidenced in the reduced spontaneous alternation behavior.

5. Conclusion

The Datura metel leaves extract distrupted motor dysfunction, reduced total locomotive activity and produced memory deficit in treated mice. These effects were reversed by naloxone suggesting involvement of opiodergic transmission system.

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7. Conflict of interest statement

The authors declare no conflict of interest.

8. Authors contribution

TA- carried out animal study and manuscript writing; UG: Carried out the extraction of the plant extract and manuscript revision; IJ: Revised the manuscript; SO- Carried out HPLC finger printing of the plant extract.

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