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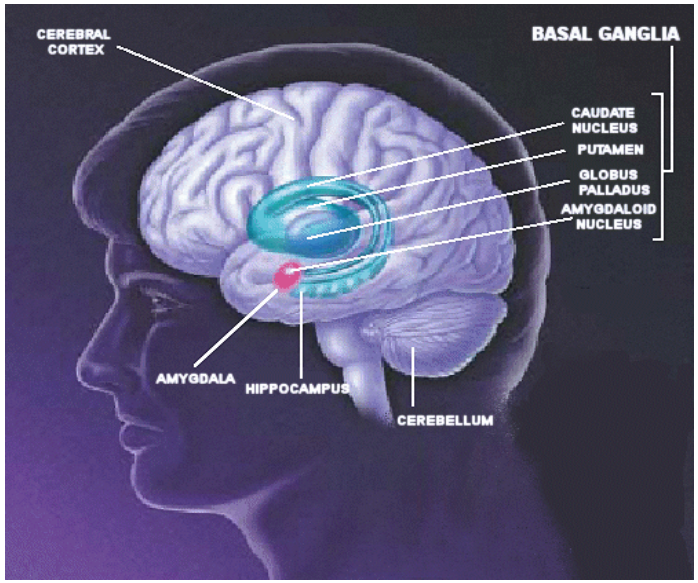
Ashenafi Girma

The effect of acute and sub-acute exposure to crude khat (*Catha edulis F.*) extract on learning and memory in rodents



Ashenafi Girma, Ephrem Engidawork.
Pharmacology and Clinical Pharmacy department,
School of Pharmacy, AAU.

Outline

Introduction

- Learning and Memory
- Khat
- Khat and Learning/memory

- Materials and methods
- Results and discussion
- Conclusion
- Recommendations

Introduction

- Learning

  - is the process of acquiring new information

- Memory

  - is the retention of the acquired information

- Memory can be classified on different criteria

  - type of memory (declarative or procedural)

  - duration of the formed memory (long term or short term)

(Kandel *et al.*, 2000)

Synaptic plasticity

- Formation of memory requires structural and other functional modifications to neurons.

  - new synapses are formed and old ones are strengthened

- patterns of neuronal activity that lead to distinct and enduring changes in synaptic strength

(Mozzachiodi and Byrne, 2008; Lombroso and Ogren, 2009)

Signaling mechanisms in LTP

- LTP has two phases

  - early phase

    . protein synthesis-independent pathway

  - late phase

    . protein synthesis-dependent **(Lu *et al.*, 1999)**

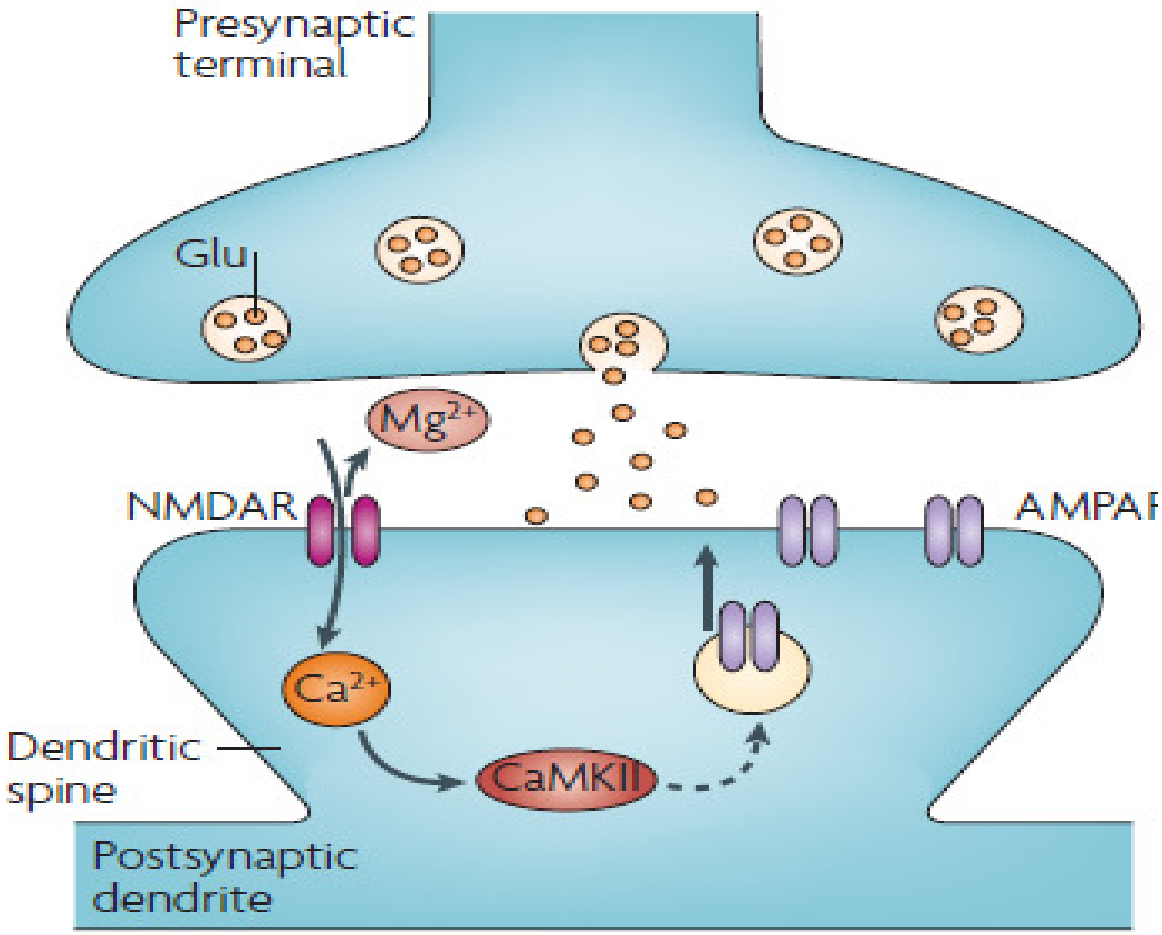
- 2 postsynaptic receptor subtypes

  - AMPA → Na+

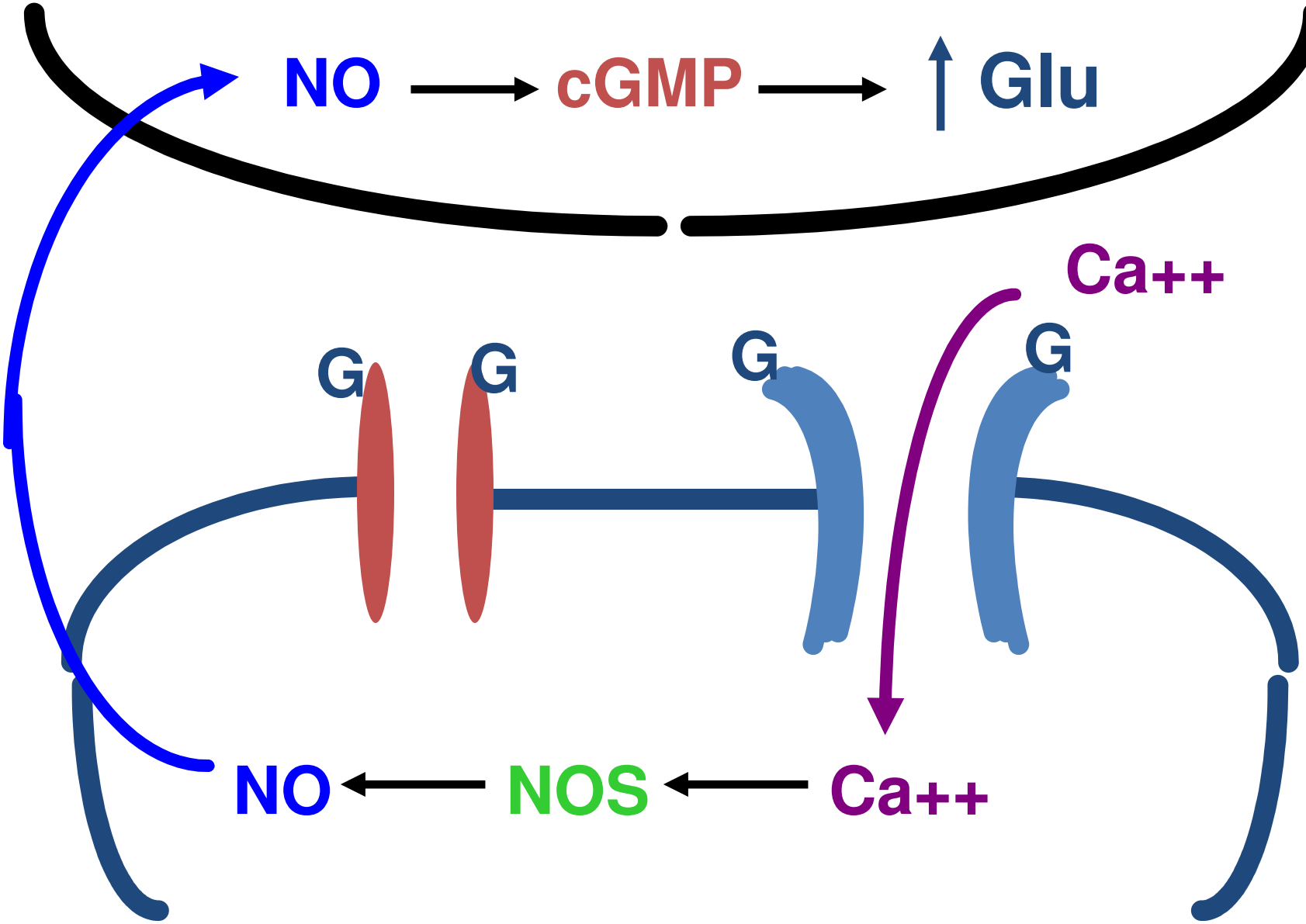
  - NMDA → Ca++

- Glu ligand for both Rs (excitatory)

NMDA dependent LTP



(Kauer and Malenka, 2007)


NO
cGMP
Glu
Ca++
G
G
G
G
NO
NOS
Ca++

L-LTP

- protein dependent pathway of potentiation
- Involves PKA and CREB
- CREB is activated by phosphorylation lead to the expression of IEG
- IEG leads to encoding of TFs that regulate expression of d/t genes to synthesize d/t proteins.

(Davis and Laroche, 1998; Sheng and Kim, 2002)

Khat

- Contains a no. of chemical constituents
  - cathinone is the main psychoactive constituent **(Kandela, 2000)**

- Chewed to attain a state of **euphoria and stimulation**, in the believe that it **would increase their level of performance**, **energy**, **alertness** and generally **to enhance their cognitive ability** **(Wilder et al., 1994)**

- Extensive study on the effect of khat use on learning and memory is lacking
  - usually deducted from studies made on other substances

Objectives

General objective

- To investigate the effect of orally administered fresh crude khat extract on learning and memory in mice.

Specific objectives

- on the ability to learn and memorize in avoiding shock in active avoidance protocol.
- on the ability to learn and memorize spatial tasks in Morris water maze paradigm.
- on spatial task solving ability in multiple T-maze protocol.

Materials and methods

Chemicals

- Diethyl ether(Sigma_Alorich^{R}, Germany)
- Chloroform (Sigma_Alorich^{R},Germany)
- Tween 80

Collection of plant material

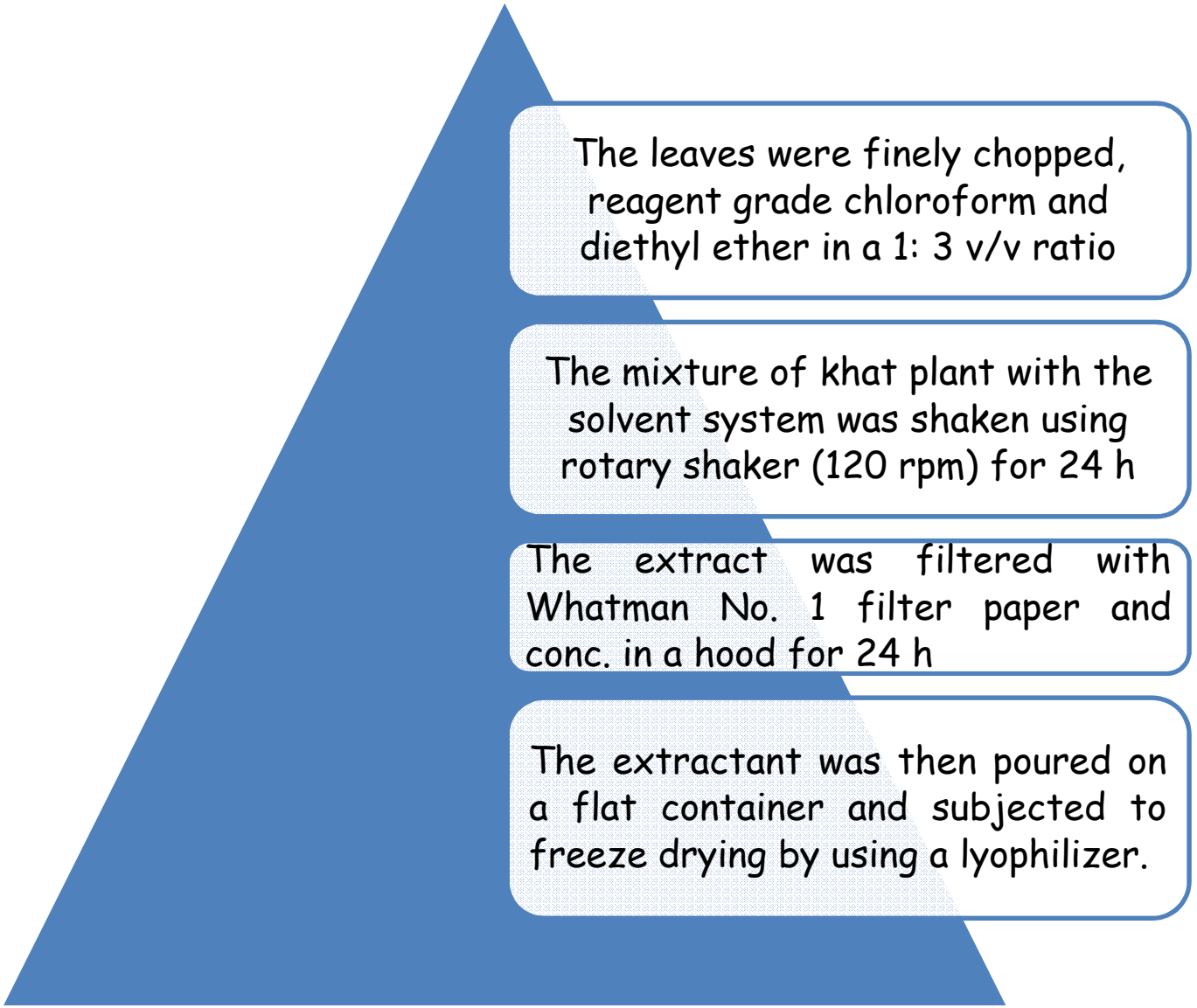
Bundles of *C. edulis F.* shoots and small branches (about 6000 g) were purchased in December 2011

The fresh bundles were packed in plastic bags and transported in an ice box

The fresh leaves were immediately kept in a deep freezer

Preparation of crude extract

(Connor *et al* ., 1999)



Experimental Animals

Adult albino mice

- 25 - 35 g
- 6- 8 weeks
- animal house of the School of Pharmacy

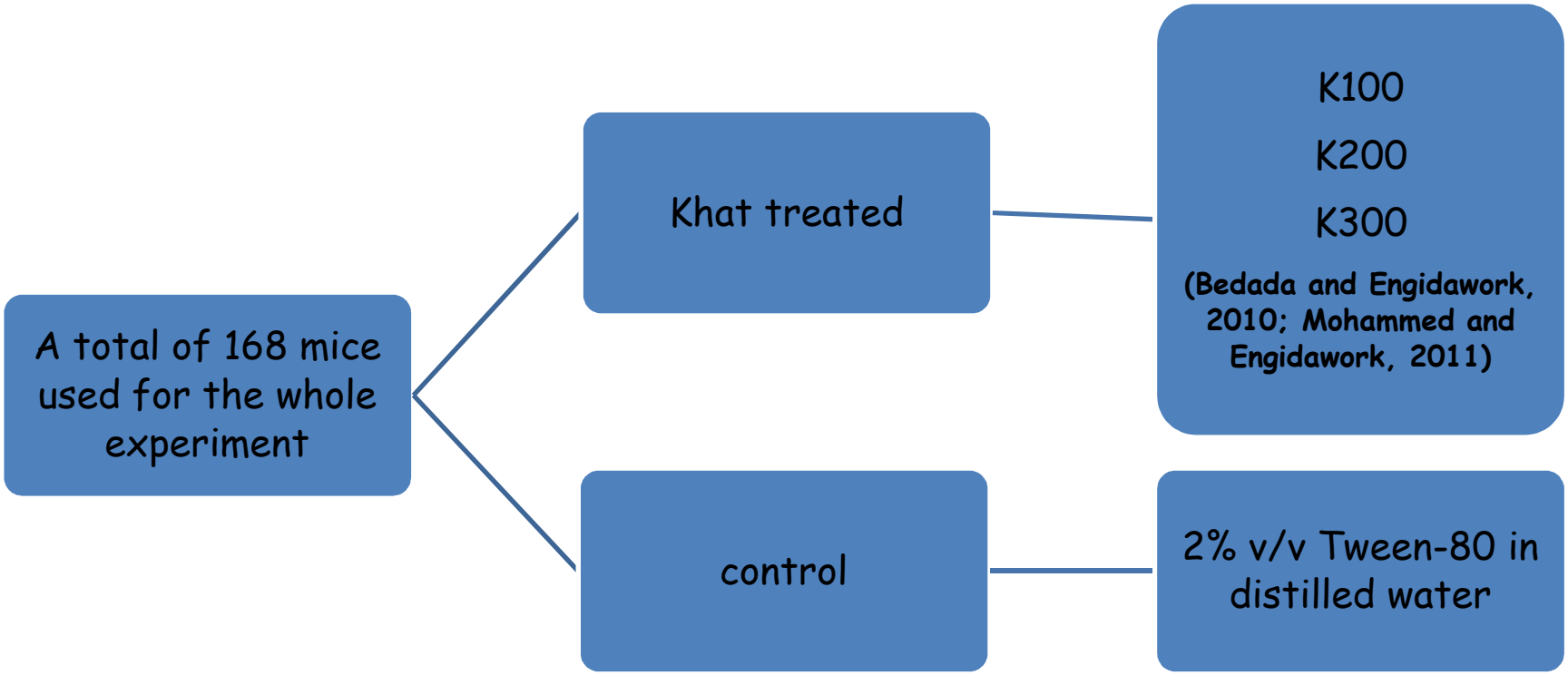
Housing and env't

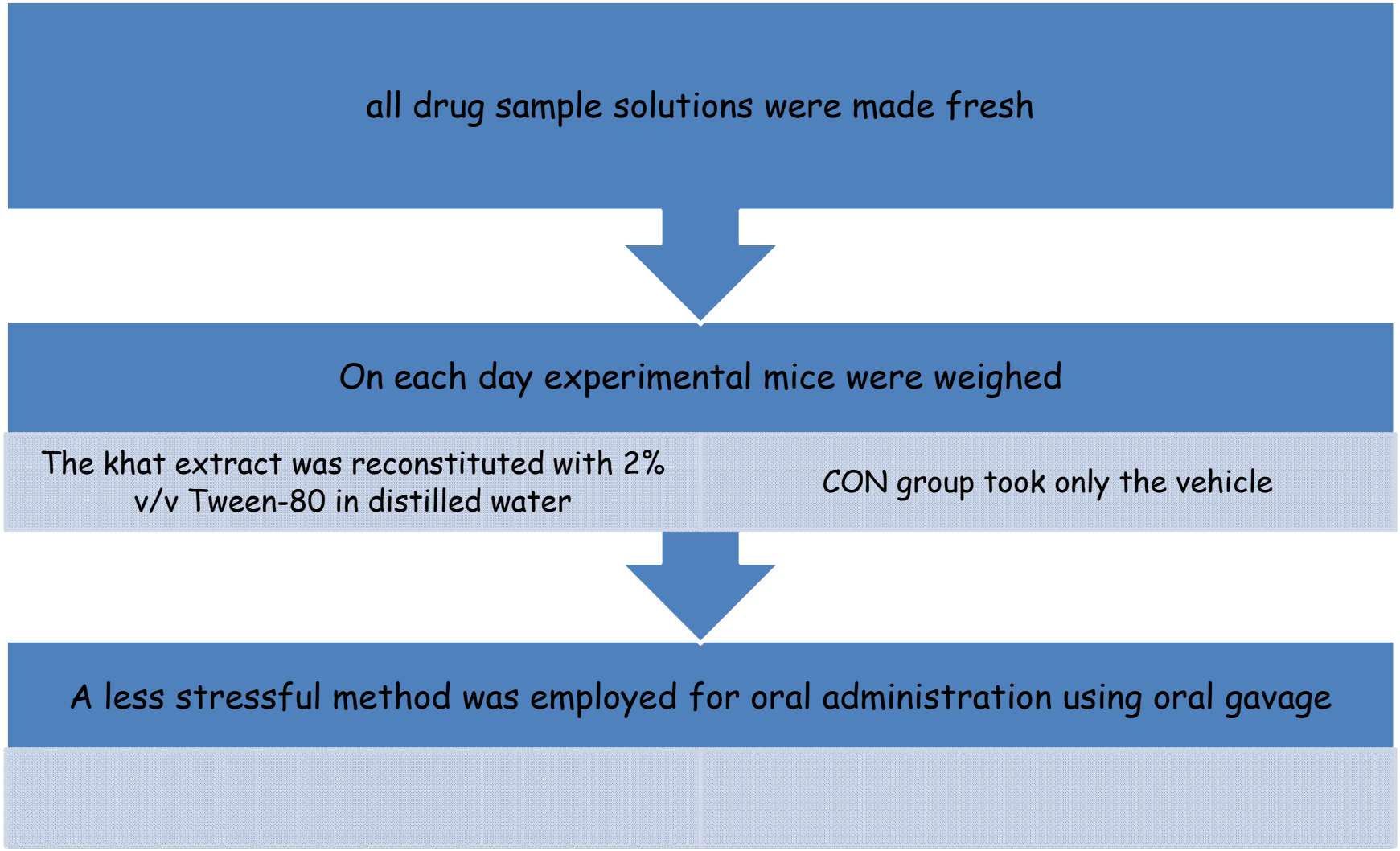
- groups of six, wooden material every 3 days
- allowed free access tap water and standard laboratory pellet
- 21±1 ◦C, 12 h/12 h light/dark cycle
- All experimental procedures on mice were done after 4:00 O'clock

care and handling

- internationally accepted standard guidelines for use of animals

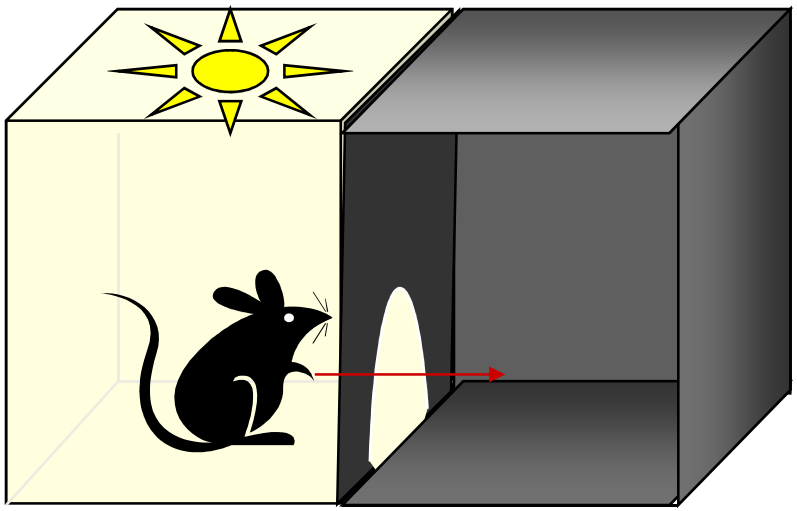
Grouping and Dosing of animals

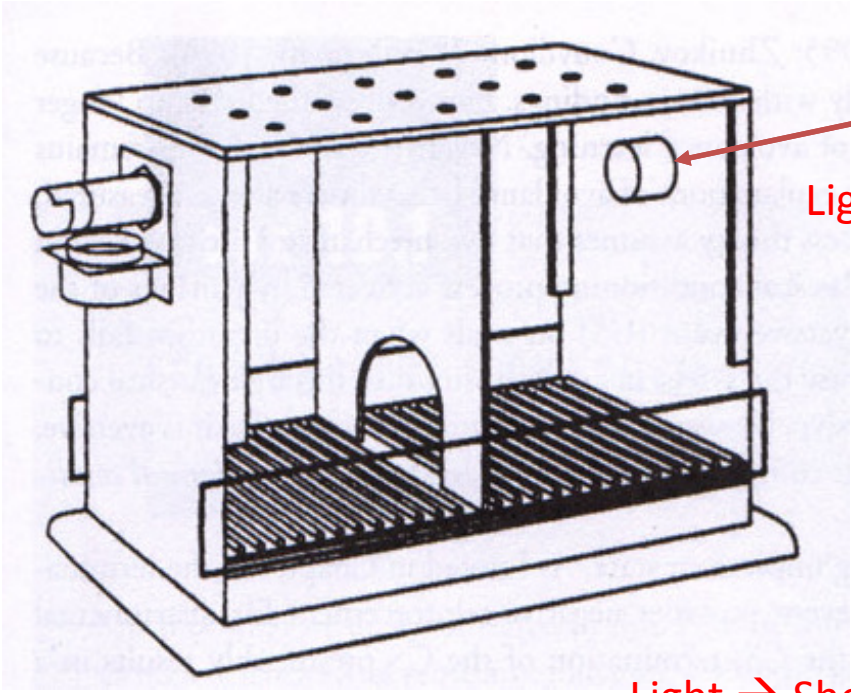



all drug sample solutions were made fresh
On each day experimental mice were weighed
The khat extract was reconstituted with 2% v/v Tween-80 in distilled water
CON group took only the vehicle
A less stressful method was employed for oral administration using oral gavage

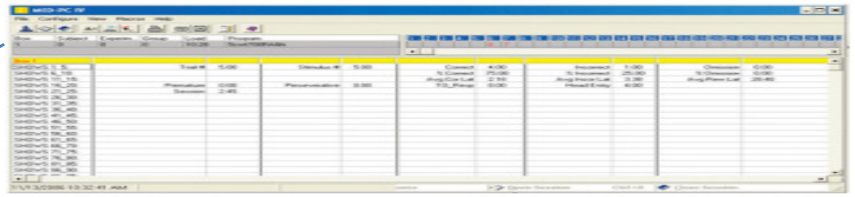
Active avoidance test

- A shuttle box apparatus (Columbus instruments (PACS-30), USA).
  - animal's location was sensed via infra-red beams located in each compartment



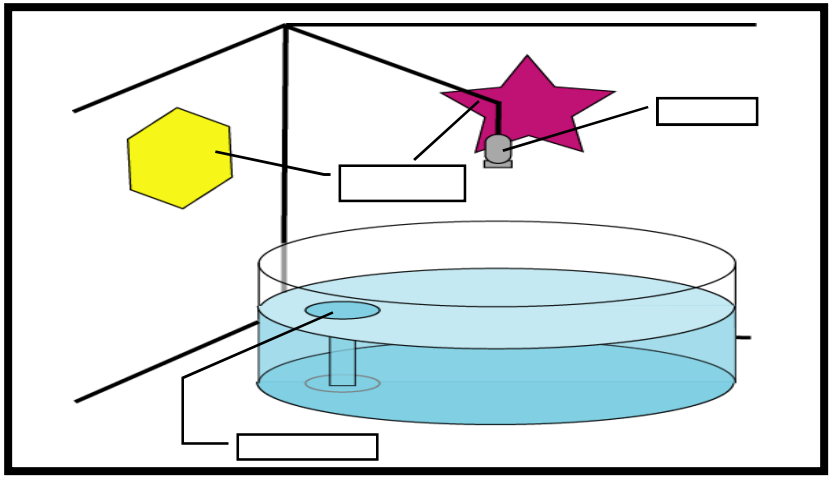


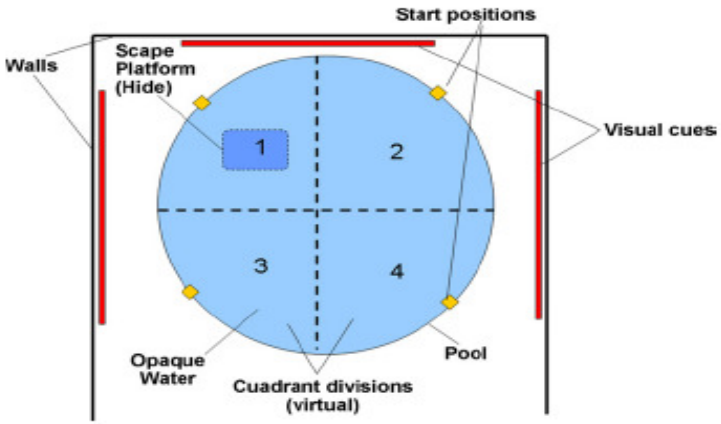
- Light (10 W, switched on alternately in the two compartments) was used as the CS.
- The US was an electric foot shock (alternating current, 0.3 mA for 9 sec)
- The CS preceded the US by 10 s and When shock occurred, the CS remained.
- A 15 sec ITI
- Each trial was classified as:



  - avoidance (crossing to the other side prior to shock onset)
  - escape (crossing to the other side after shock had started)
  - null (remaining in the original compartment and receiving 9 s of shock)

Morris water maze test





Morris water maze (MWM)

- circular pool (150 cm diameter, 50 cm depth)
- filled with tap water up to 25 cm high
- hidden platform (1 cm beneath water surface)
  - ✓ 10 cm × 24 cm
  - ✓ Only identified using distal extra-maze and intra-maze cues with different colors and dimensions attached
  - ✓ the middle of the NE quadrant and remained in the training days

• Water temperature was maintained at 22±1°C

- spatial acquisition phase consisted of 16 training trials: 4 x 4 with an inter-trial interval of 5 min

- Mice were released randomly with their heads facing the pool wall (N, W, S, and E)

- allowed to swim and search for the platform for 90 seconds
- The latency to reach the hidden platform was recorded using a video camera

- Finding the platform was defined as staying on it for at least 3 sec

- On 6th day subjects received a probe trial
  - ✓ the platform was removed
  - ✓ mice were released from the S start point
  - ✓ were allowed to swim freely for 90 seconds
  - ✓ time spent in target quadrant was recorded
- Mice were not trained during the time periods between 4th and 6th day.

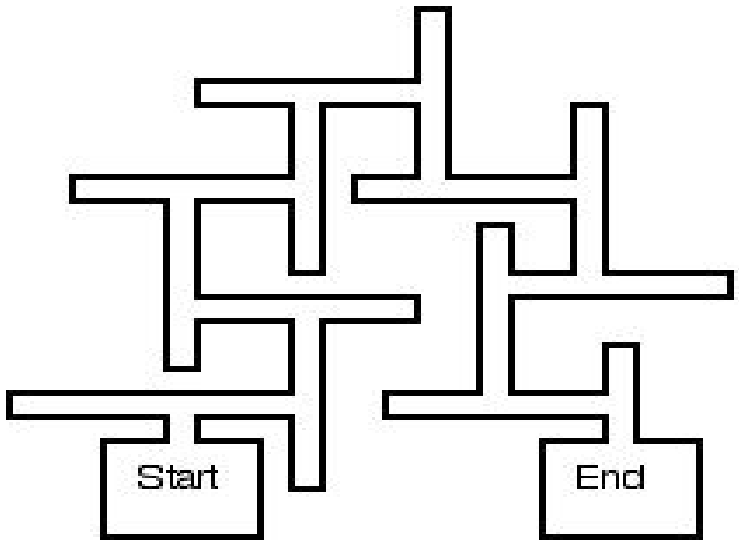
Multiple T-maze test

- Multiple T-maze

  - constructed of wood, with seven choice points

  - dimensions (150 cm × 130 cm × 15 cm) and

    a path width of 8 cm.



- mice were deprived of food for 16 h and were placed in a start box
- the trial was completed when mice had reached the goal box or, if failed, after 5 min

- Immediately after each trial, the entire maze was cleaned with dilute alcohol solution

- trained with 3 trials per day for 4 days
- trials were recorded using video camera
- the following parameters were measured, wrong decisions and latency to reach the goal box.

- On 6th day subjects received a probe trial
  - were allowed to the maze for 5 min
  - time to reach the goal and wrong decisions were recorded

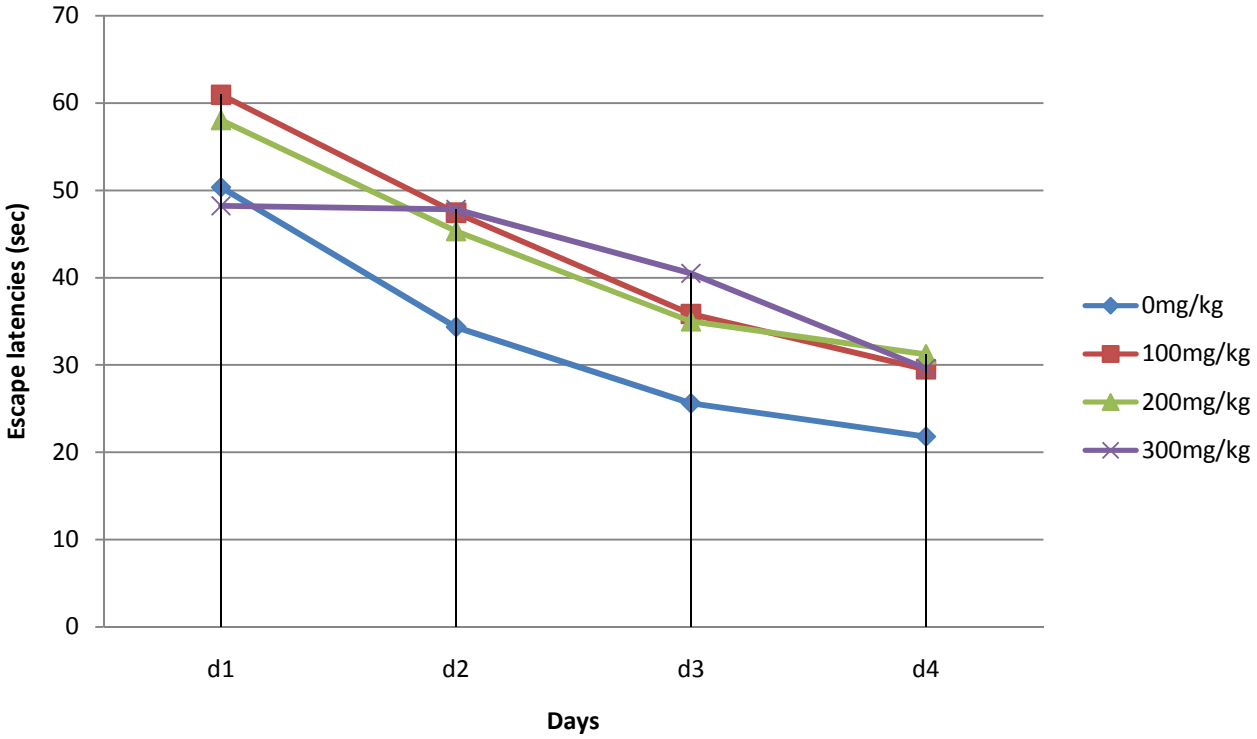
Statistical analysis

- ✓ Data Analysis was performed using SPSS, version 16.0
- ✓ All data were expressed as mean ± SEM
- ✓ One way ANOVA
- ✓ Paired sample t-test
- ✓ General linear model: repeated measures of ANOVA
- ✓ Significant differences were set at $p < 0.05$

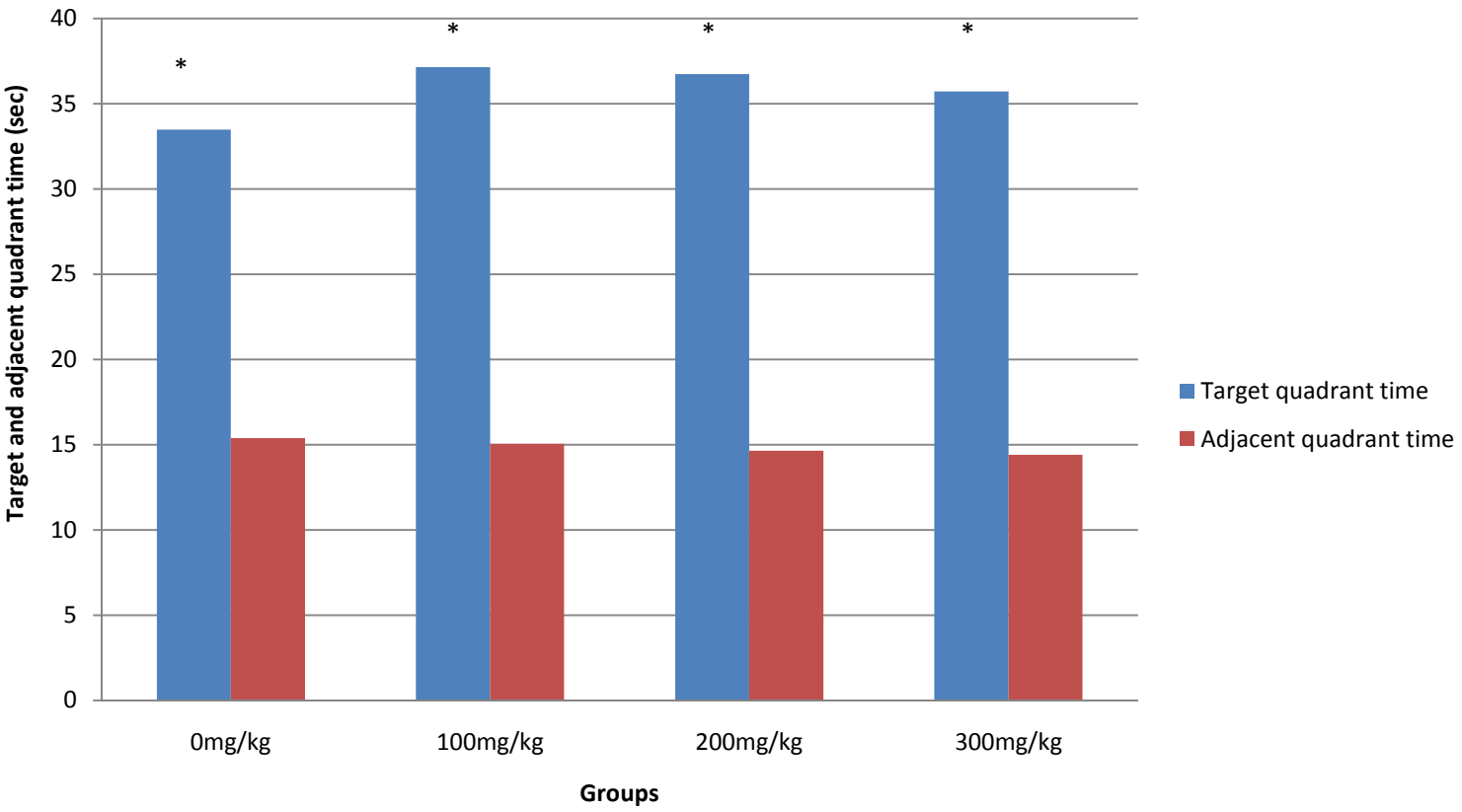
RESULTS

water maze performance (*acute*)

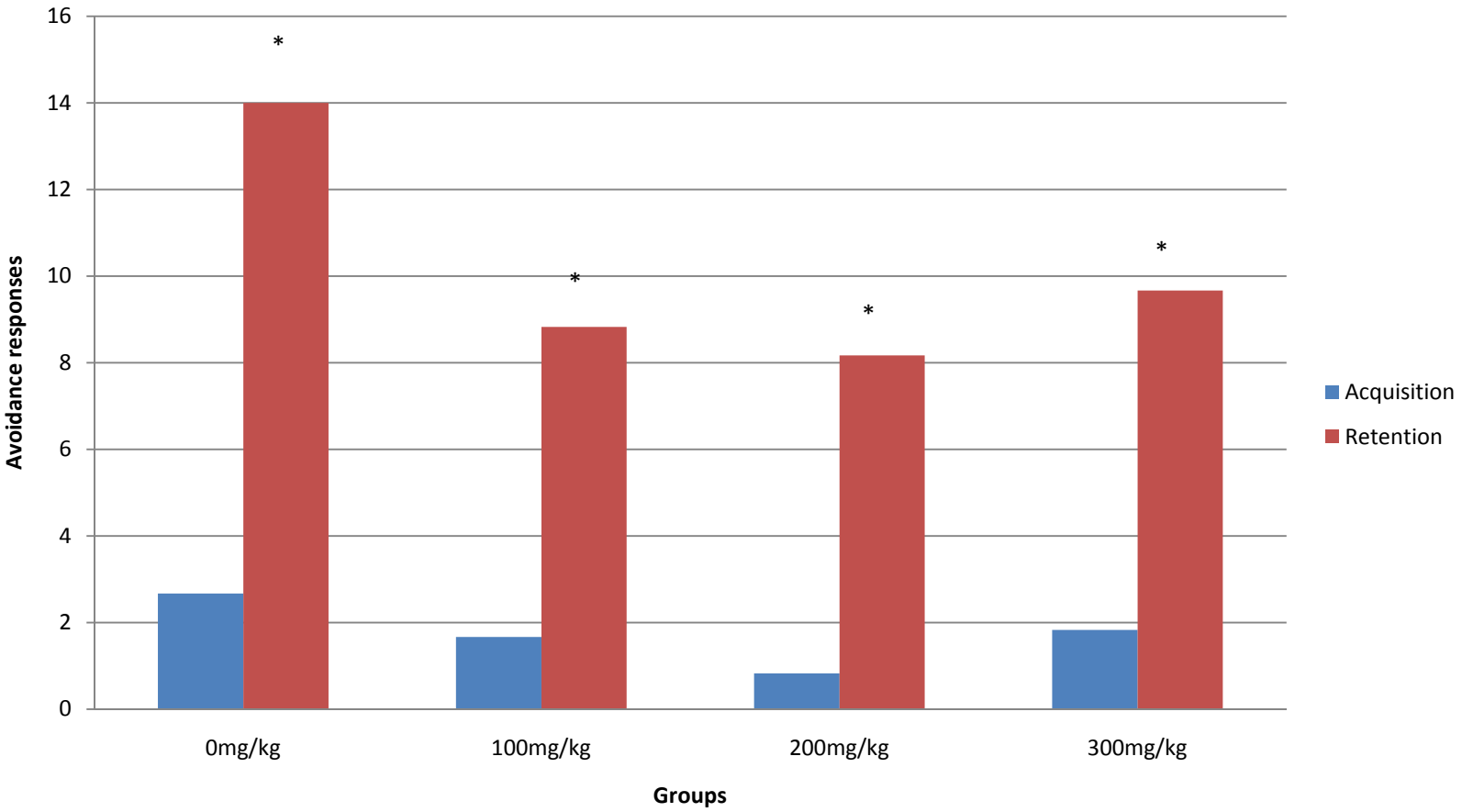
Acquisition training



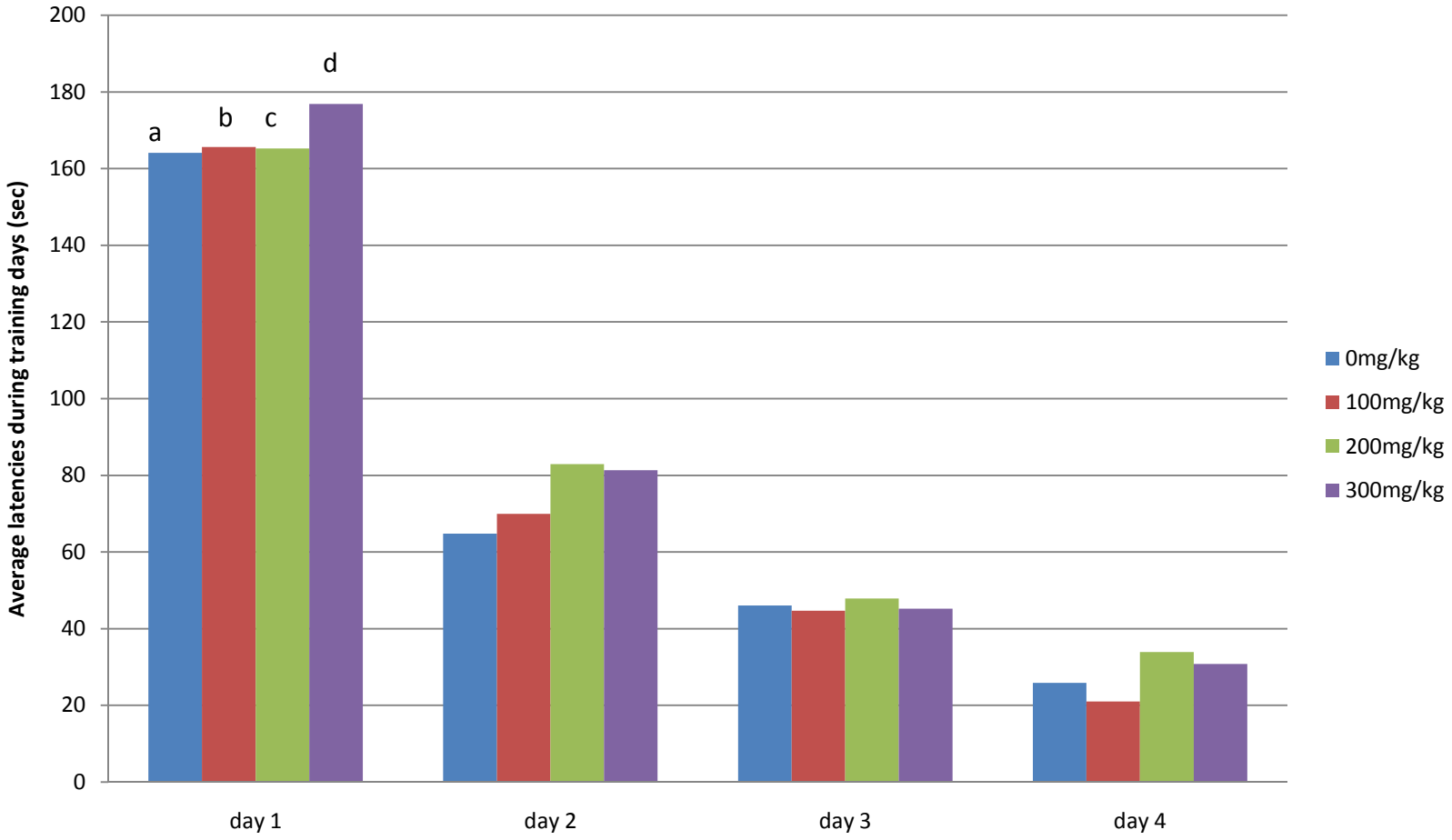
Probe trial

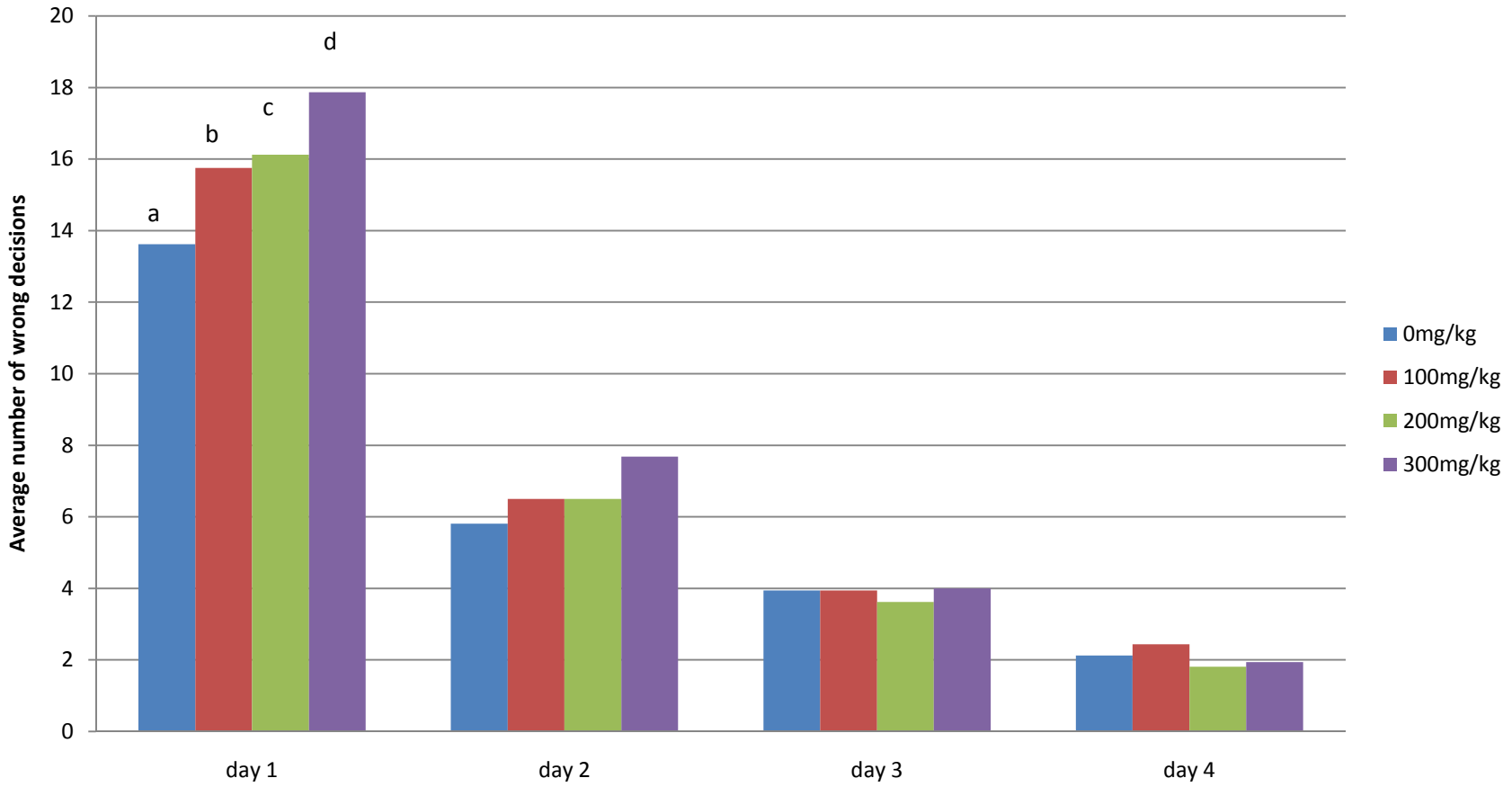

40
35
30
25
20
15
10
5
0
*
*
*
*
Target and adjacent quadrant time (sec)
0mg/kg
100mg/kg
200mg/kg
300mg/kg
Groups
Target quadrant time
Adjacent quadrant time

Active avoidance performance (*acute*)

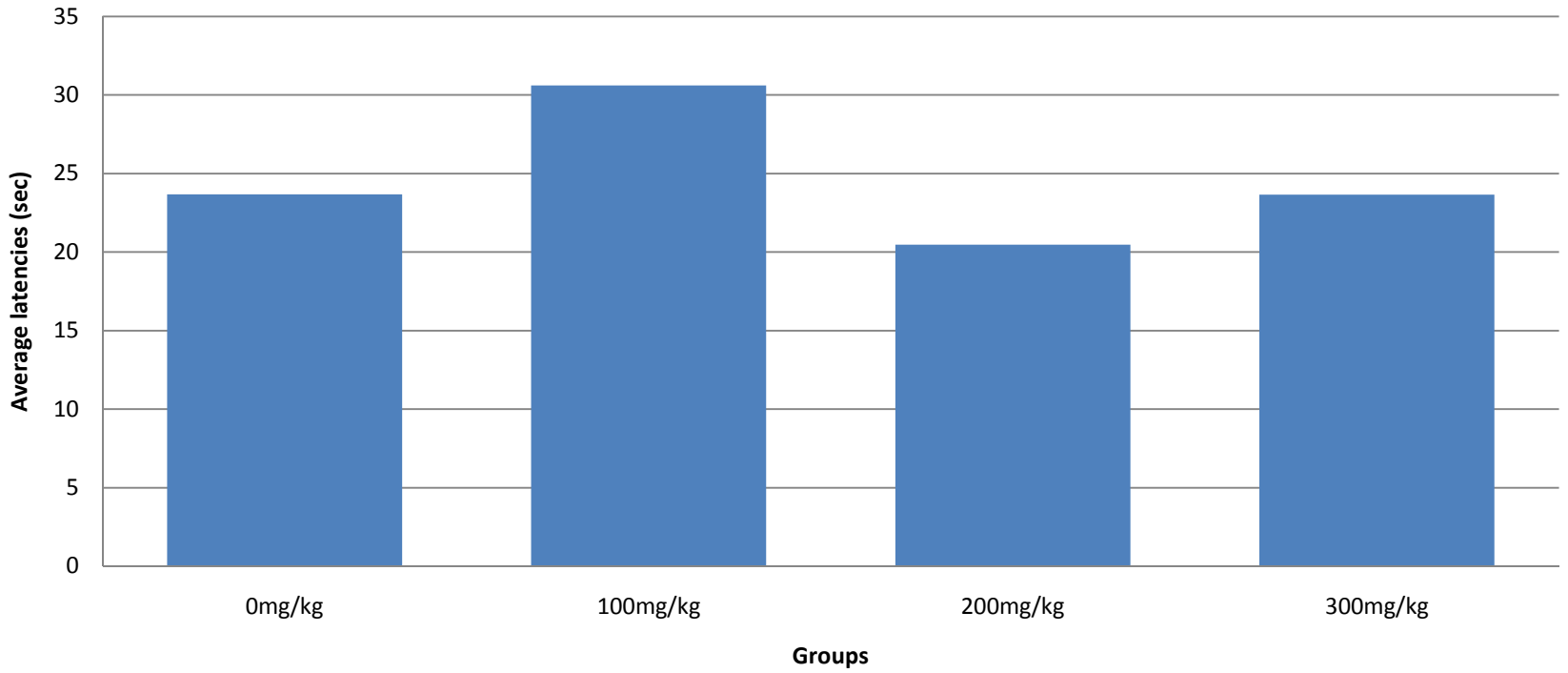


Multiple T-maze performance *(acute)*



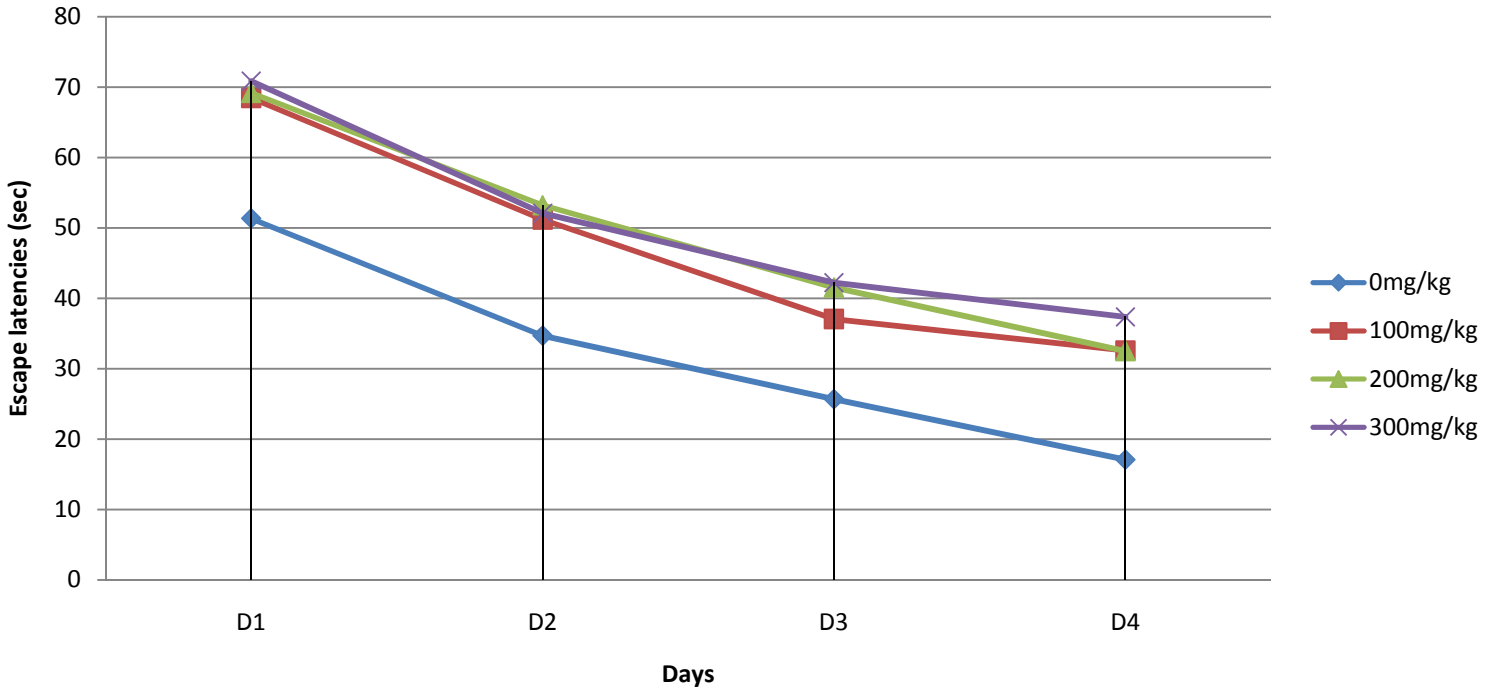

Average number of wrong decisions
20
18
16
14
12
10
8
6
4
2
0
a
b
c
d
day 1
day 2
day 3
day 4
0mg/kg
100mg/kg
200mg/kg
300mg/kg

Probe trial

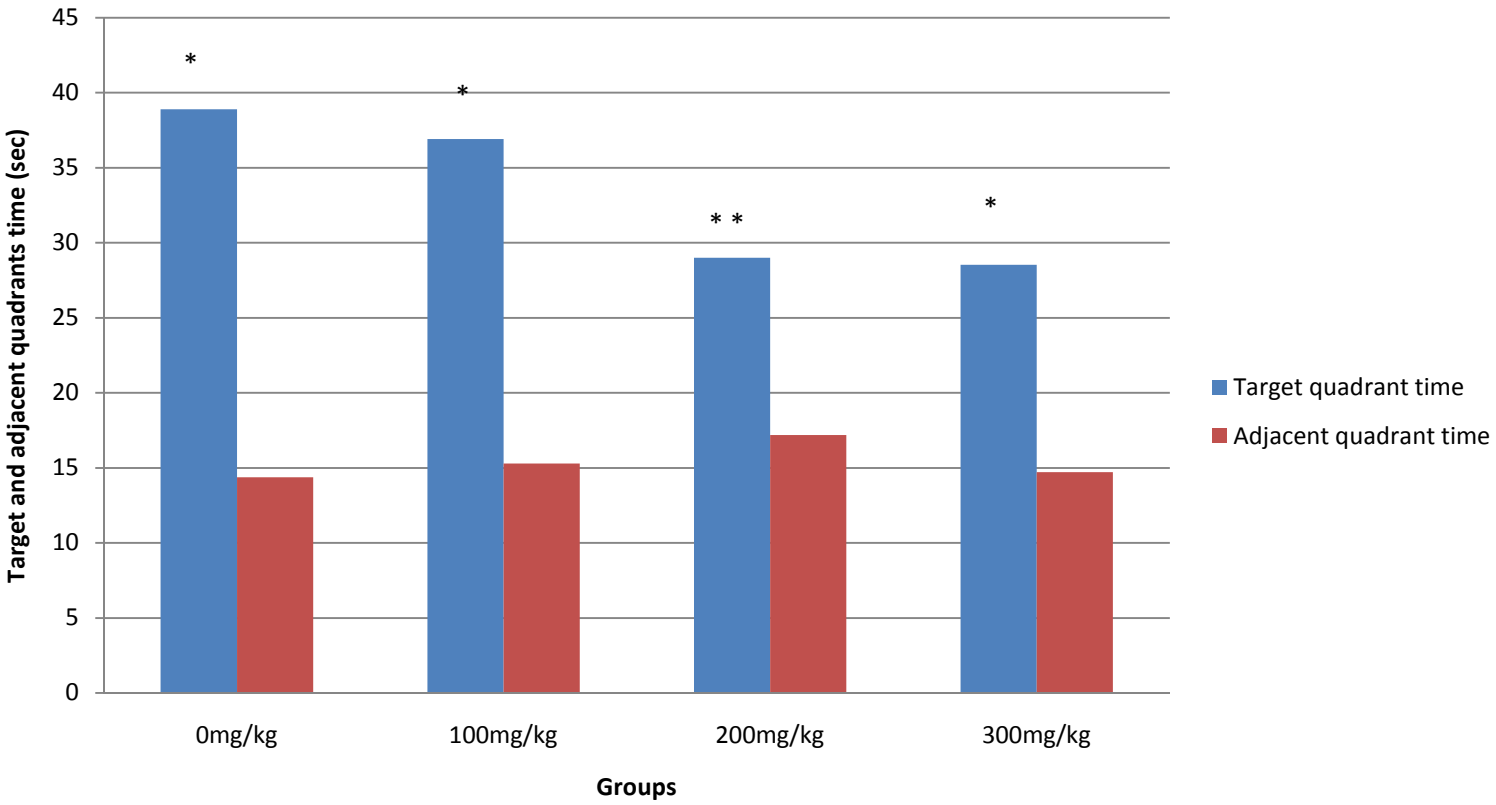

35
30
25
20
15
10
5
0
Average latencies (sec)
0mg/kg
100mg/kg
200mg/kg
300mg/kg
Groups

water maze performance *(sub-acute)*

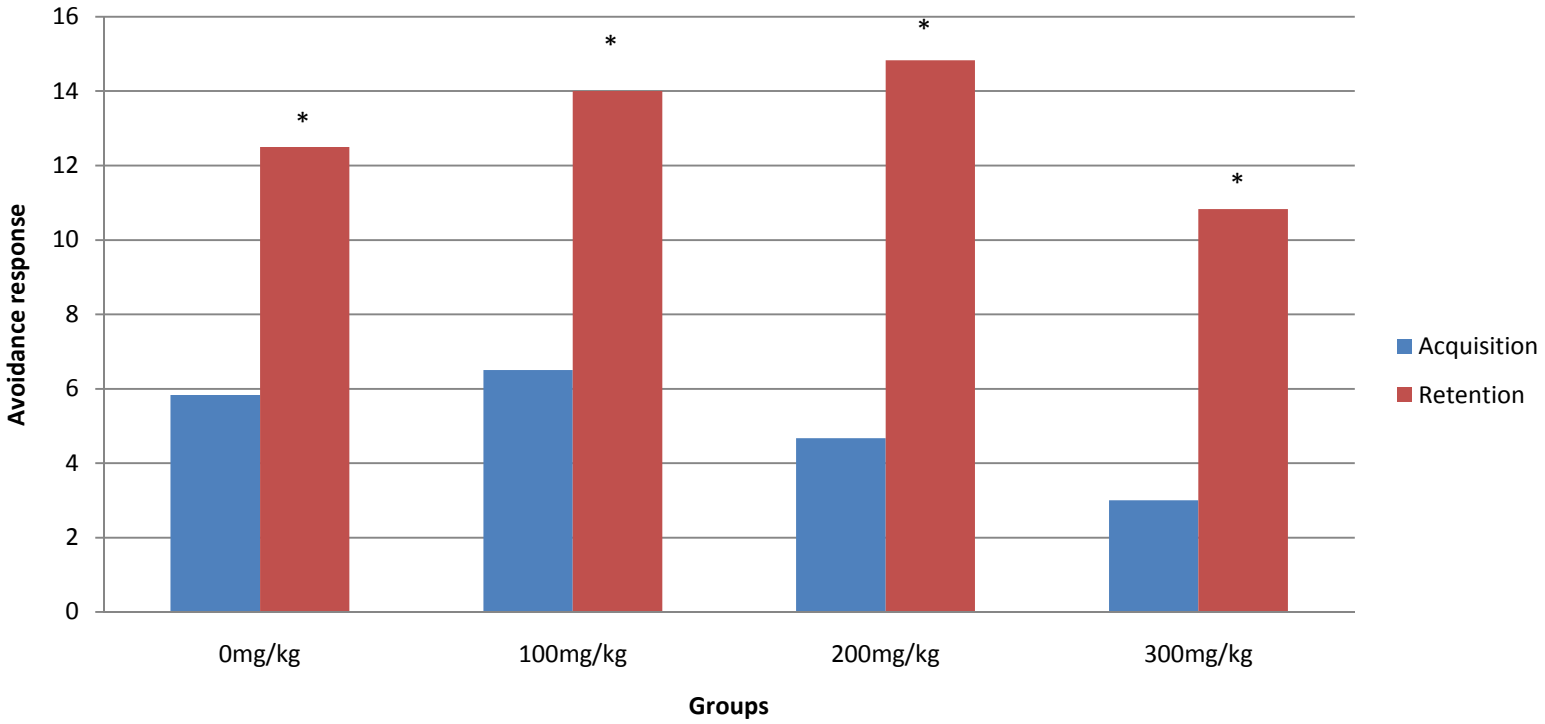
Acquisition training



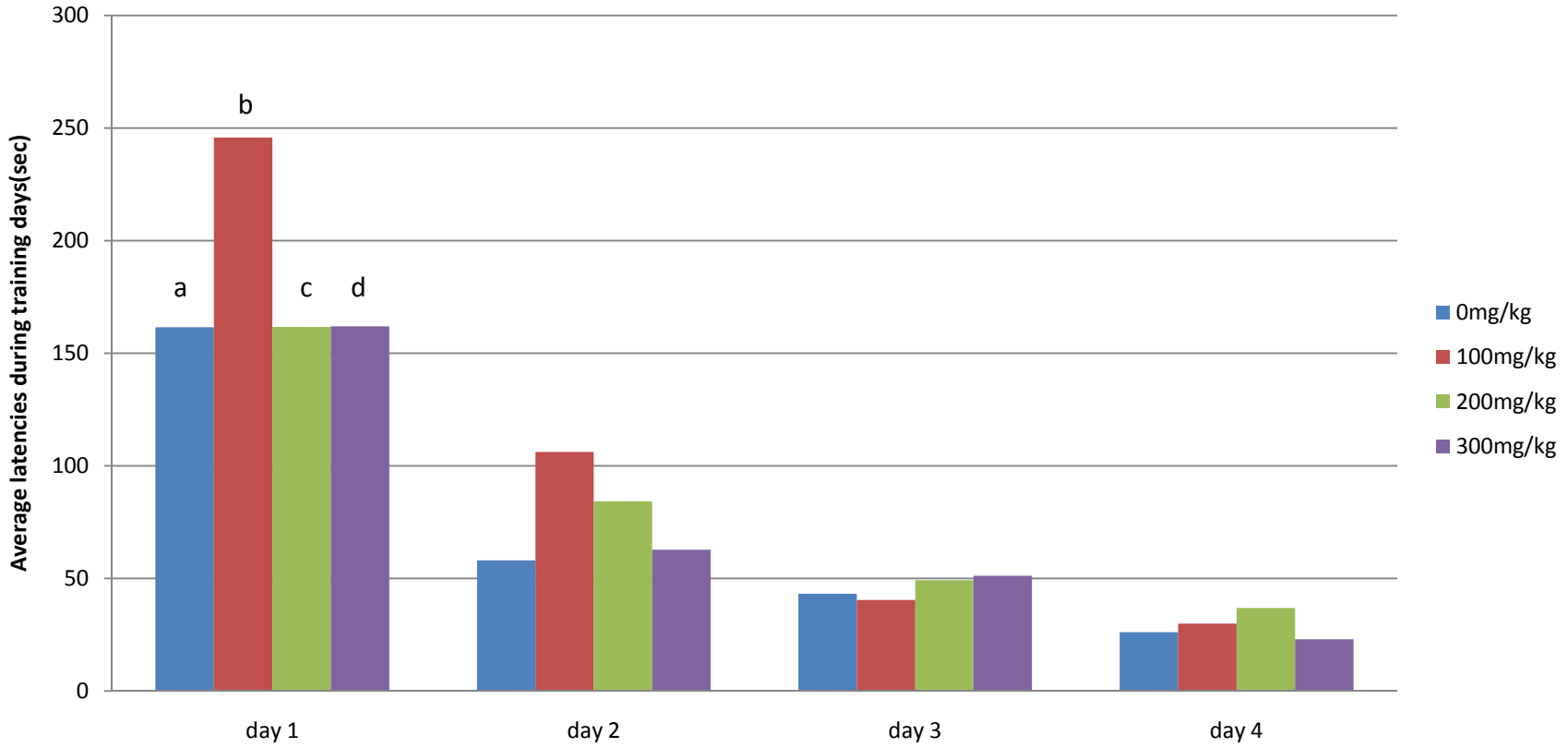
Probe trial

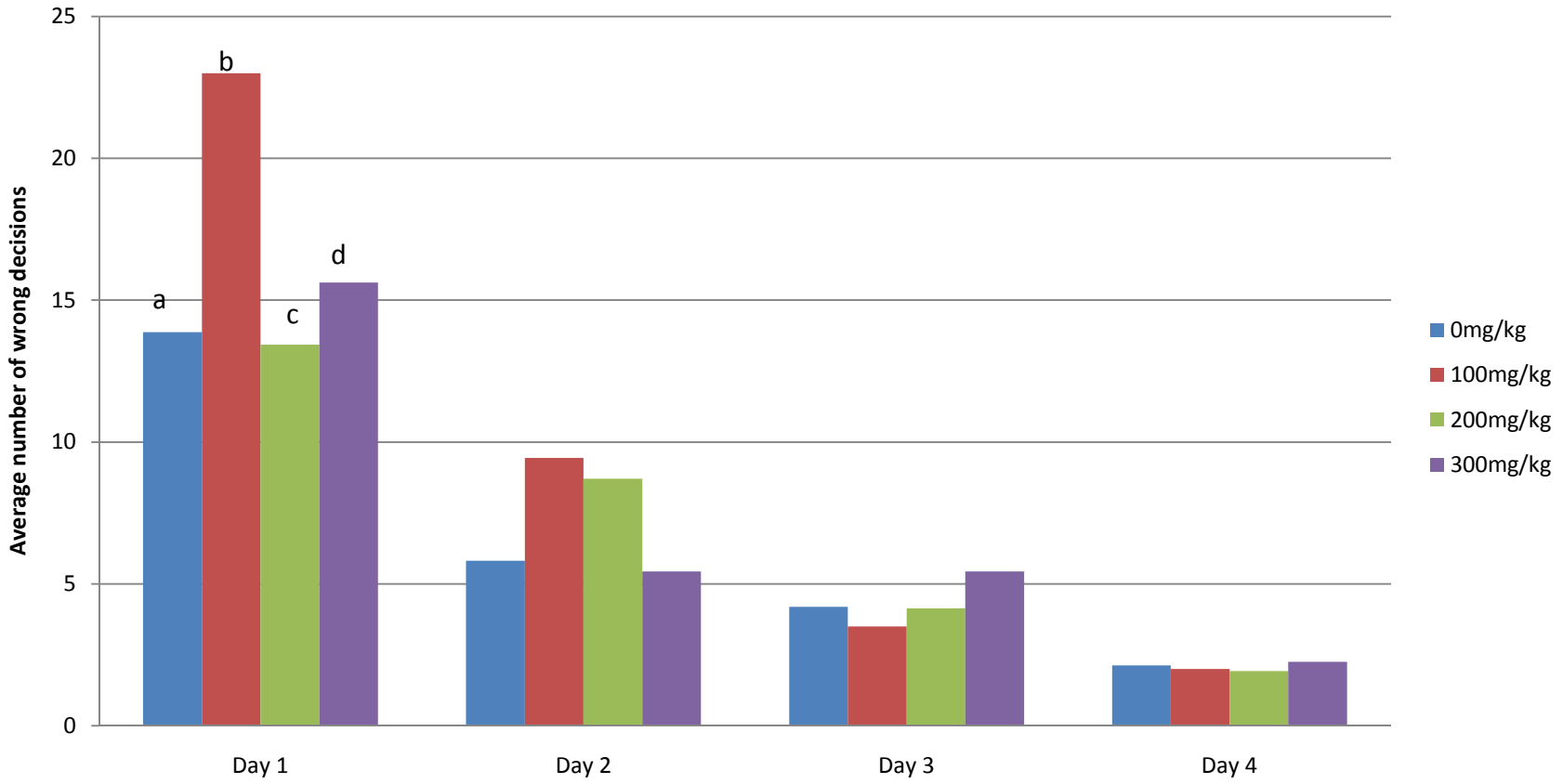

45
40
35
30
25
20
15
10
5
0
Target and adjacent quadrants time (sec)
*
*
* *
*
0mg/kg
100mg/kg
200mg/kg
300mg/kg
Groups
Target quadrant time
Adjacent quadrant time

Active avoidance performance (*sub-acute)*

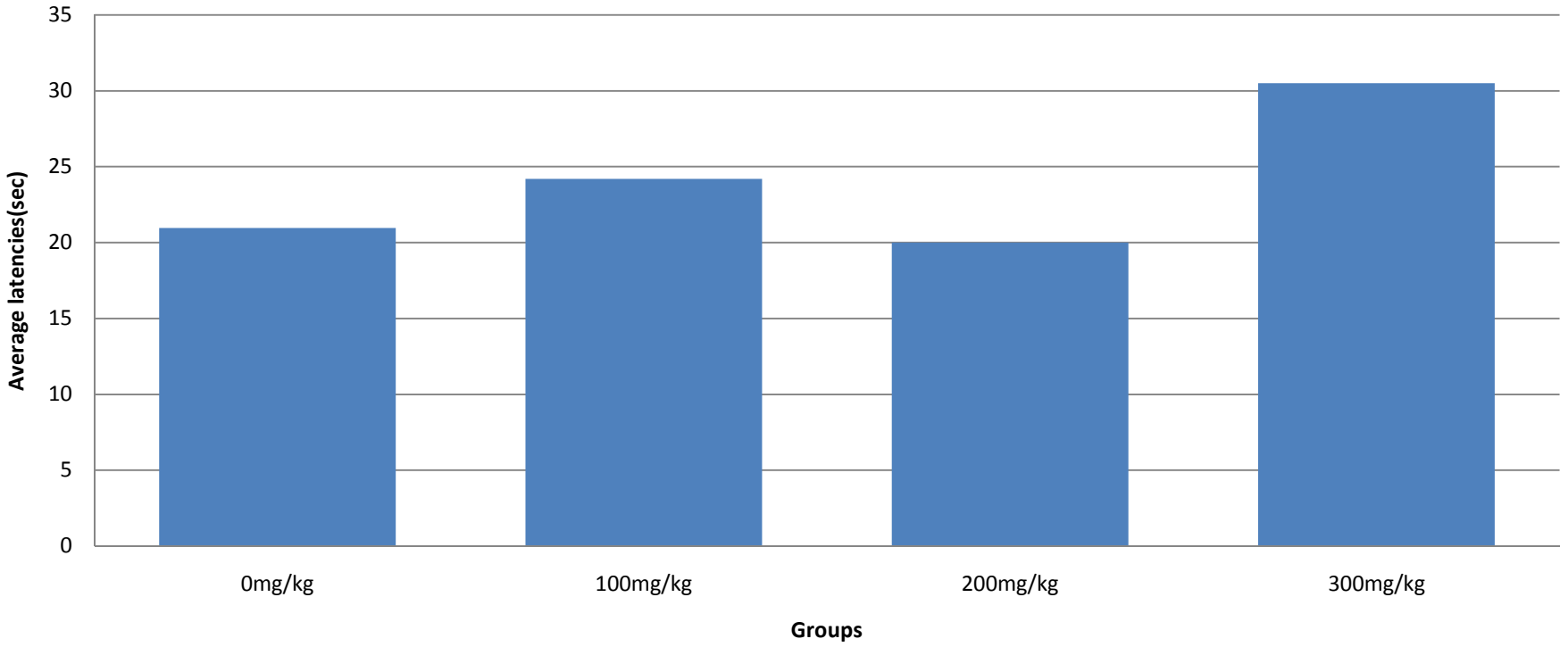


Multiple T-maze performance *(Sub-acute)*




Average number of wrong decisions
25
20
15
10
5
0
a
b
c
d
Day 1
Day 2
Day 3
Day 4
0mg/kg
100mg/kg
200mg/kg
300mg/kg

Probe trial


35
30
25
20
15
10
5
0
Average latencies(sec)
0mg/kg
100mg/kg
200mg/kg
300mg/kg
Groups

Discussion

- Cathinone acts by releasing catecholamines from presynaptic storage sites and subsequently inhibit their uptake,
  - dopamine, serotonin and noradrenaline **(Kimani *et al.*, 2008)**

Dopamine

- The hippocampal *CA1* region receives dopaminergic input from midbrain sources
  - An increase in the hippocampal dopaminergic function improves learning in animals **(Packard and White, 1991)**
  - In the experiment learning in dopamine-deficient mice had failed to perform learning in water escape **(Victor *et al.*, 2004)**

Serotonin ??

- Inhibition of the system enhances performance **(Barnes *et al.*, 1990, 1992)**.
- Studies in humans have also shown increased 5-HT neurotransmission impaired spatial working memory **(Luciana and Collins, 1998)**.
- Antagonistic effects of 5-HT towards facilitatory effects of dopamine in a wide range of behavior **(Kimani *et al.*, 2008)**.

- The present result can be explained by the effects of dopamine and serotonin on receptors seen to be cancelled each other.
  - cathinone induce the release of CNS DA and 5-HT without a net dopaminergic or serotonergic effect on learning and memory.

Conclusion

- This study showed acute and sub-acute oral administered crude khat extract with different doses had no significant effect on learning and memory in the following protocols: Morris water maze task, active avoidance conditioning and multiple T-maze task.

Recommendations

- The effects of crude khat extract on learning and memory using other paradigms needs to be investigated.
- Neurotransmitters level (dopamine, serotonine and noradrenaline) need to be determined

Contact address

- ashetefera@gmail.com
- Ephrem.engidawork@gmail.com, ephrem.engidawork@aau.edu.et

My next research plan

- **To determine the effect of khat plant on the level of dopamine and serotonin in rat brain and to elucidate the mechanism in the behavioral effect of the plant.**

Thank You!

Meet the eminent gathering once again at

Addiction Therapy-2015

Florida, USA

August 3 - 5, 2015

Addiction Therapy – 2015 Website:

addictiontherapy.conferenceseries.com