Laboratory models of high dilution-dynamization effects: Rodent models of anxiety

Paolo Bellavite, University of Verona

1. Introduction and literature review
2. Materials and Methods
3. Results and discussion

The figures are downloadable at: www.paolobellavite.it

Homeopathy for anxiety and anxiety disorders: a systematic review of the research

Pilkington,K., G.Kirkwood, H.Rampes, P.Fisher, and J.Richardson. 2006. Homeopathy 95:151-162.

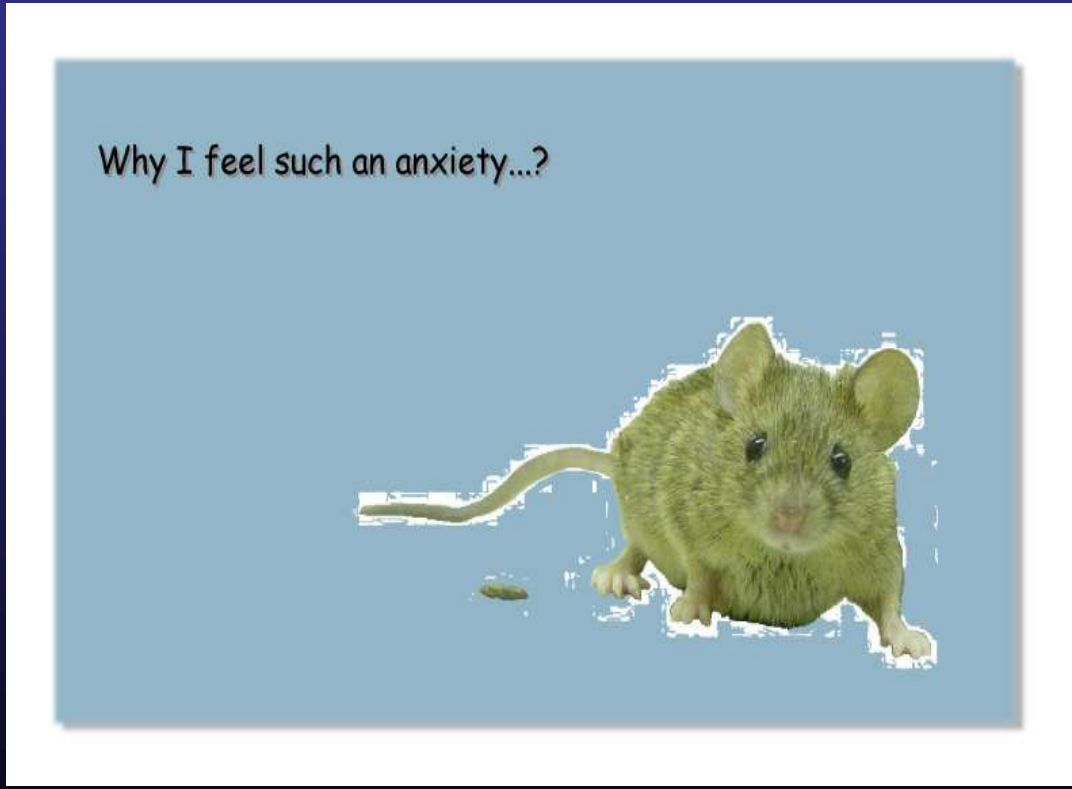
- Surveys suggest that homeopathy is quite frequently used by people suffering from anxiety. **The evidence on the benefit of homeopathy in anxiety and anxiety disorders is limited.**

Further research is necessary:

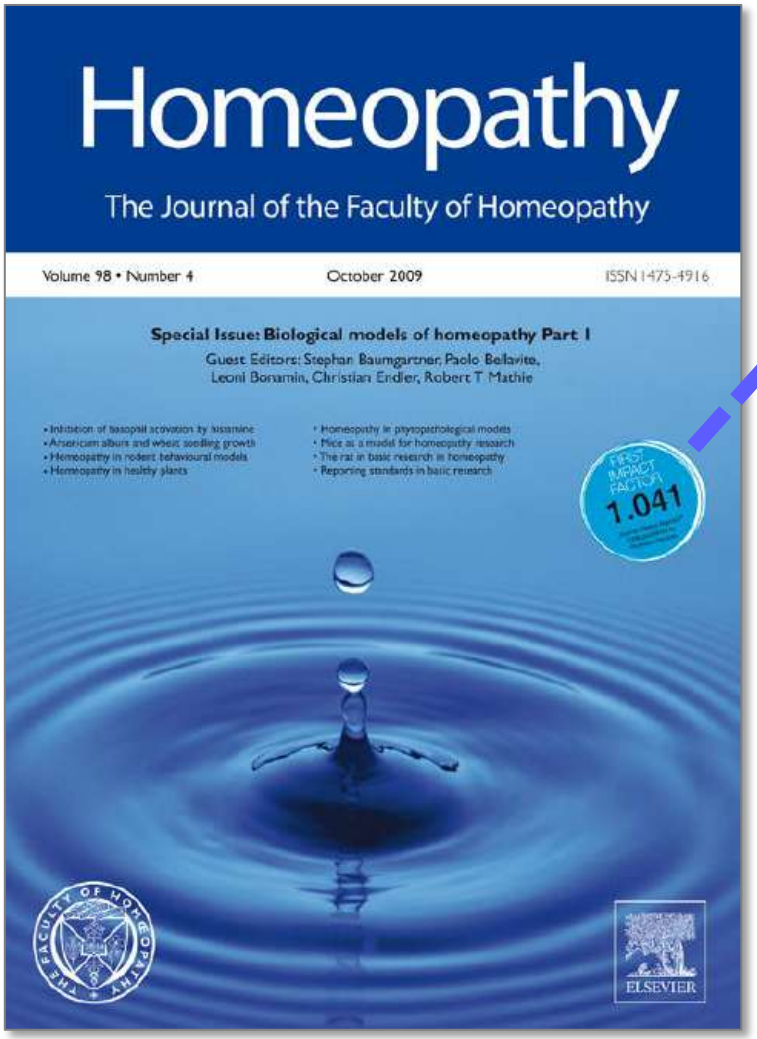
- Observational studies
- Randomized trials
- BASIC RESEARCH (in vitro, laboratory, action mechanism of drugs)

Animal models- Background

- Research in anxiety and psychopharmacology has a long history of development of animal models.
- The measurement of anxiety-related behaviour in animal models is based on the assumption that anxiety in animals is comparable to anxiety in humans.



RODENT MODELS OF ANXIETY AND PYCHOPATHOLOGY



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Homeopathy (2009) 98, 208–227
© 2009 The Faculty of Homeopathy
.2009.09.005, available online at http://www.sciencedirect.com

ORIGINAL PAPER

Assays of homeopathic remedies in rodent behavioural and psychopathological models

Paolo Bellavite[1,*], Paolo Magnani[1], Marta Marzotto[1] and Anita Conforti[2]

[1]*Department of Morphological Biomedical Sciences, University of Verona, Italy*
[2]*Department of Medicine and Public Health, University of Verona, Italy*

Reports on psychopathological and behavioural models of homeopathy in rodents - 1st part of 2

Date Author	Animal	Model	Remedy	Route	Main effects
1979 Binsard	Mouse	4 plates	Ignatia and Gelsemium 3CH,4CH,5CH	i.p. 3 weeks	Anxiolytic (Ignatia 3CH and Gelsemium 5CH only) or sedative (Ignatia 5CH)
	Rat	Staircase	Ignatia and Gelsemium 3CH,4CH,5CH	i.p. 3 weeks	Sedative (Ignatia 4CH and Gelsemium 3CH and 5CH only)
1980 Binsard et al.	Mouse	4 plates Escape test Rota-rod	Gelsemium 3CH, 5CH, 7CH	i.p. 3 weeks	Gelsemium 3CH reduces exploration, Gelsemium 7CH increases it
1981 Guillemain et al.	Mouse	Strychnine. Induced convulsions	Ignatia 3D, 3,5,7,12CH	i.p. 0.5 ml/20g single dose	Slight protective effect of 3DH and 5CH
1986 Sukul	Rat	Cataleptogenic effects of restraint	Gelsemium, Cannabis, Graphites and Agaricus Muscarius (30CH and 200CH)	Per os	Increase cataleptogenic effects of restraint.
1991 Sukul et al.	Rat (and cats)	Electrophysiology of SNC	Arnica 30CH, Hypericum 200CH, Arsenic 30CH	Per os (0.5 ml)	Arnica and Hypericum decrease firing rate, Arsenic increase it.

Reports on psychopathological and behavioural models of homeopathy in rodents - 2nd part of 2 (** non-homeopathic journal)

Date Author	Animal	Model	Remedy	Route	Main effects
1997 Cristea et al.	Mouse	Behavioural tests	Chamomilla 5CH and 30CH	Per os 4 times/day for 1 day (5CH) or 2 times/day for 3 days (30CH)	Stimulating effects with 5CH and tranquillizing effects with 30CH
1999-2001 Sukul	Mouse	Loss of righting reflex due to ethanol	Nux vomica 30CH	Per os 0.05 ml/2 ml water and given at 0.05 ml/individual.	Protective effect
2001 Bousta et al. **	Mouse	Electric stress Light-dark test Blood cell count Gastric lesions	Atropa belladonna Gelsemium sempervirens Poumon histamine	I.p. 30 min before stress and test	Reversal of stress-induced alterations
2005 Ruiz-Vega	Rat	Sleeping behaviour	Coffea cruda 30CH and 200CH	Per os in feeding bottle	Coffea 30CH changes spectral power of EEG Delta band
2008 Da Silva Rocha	Rat	Open field	Rhus toxicodendron 200CH	Per os 24 h	Decreases locomotion in hyperactive rats
2008 Pinto	Mouse	Open field Forced swimming	Chamomilla 6CH	Per os 7days	Prevents decrease of general activity. In O.F.

Animal models-Our objectives

1. **To set up validated models in animal models of anxiety-behavior (and should allow publication in major intnl journals!)**

2. **To test the effects of several homeopathic medicines used in anxiety in humans (5CH)**

3. **To identify one or more active compounds and to test several dilutions/dynamization in rigorous reproducible way (4-5-7-9-30 CH)**

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DRUGS

1. **Homeopathic drugs were provided by Boiron Laboratories (Lyon) in 30% hydroalchoolic solution.**

2. **Just before starting treatments, the solutions were 100-folds diluted in distilled sterile and apyrogenic water, then vigorously succussed by hand, thus lowering the alcohol concentration to 0.3 %.**

3. **All solutions were delivered by intraperitoneal (i.p.) injection, 0.3 ml/mice.**

Dilution and dynamization

0.4 ml **Gels 3CH** + 39.6 ml H_20 → *shaking* → 40 ml **Gels 4CH**

0.4 ml **Gels 4CH** + 39.6 ml H_20 → *shaking* → 40 ml **Gels 5CH**

0.4 ml **Gels 6CH** + 39.6 ml H_20 → *shaking* → 40 ml **Gels 7CH**

0.4 ml **Gels 8CH** + 39.6 ml H_20 → *shaking* → 40 ml **Gels 9CH**

0.4 ml **Gels 29CH** + 39.6 ml H_20 → *shaking* → 40 ml **Gels 30CH**

0.4 ml **EtOH 30%** + 39.6 ml H_20 → *shaking* → 40 ml **EtOH 0.3%**

0.4 ml **EtOH 30%** + 39.6 ml H_20 → *shaking* → 40 ml **EtOH 0.3%**

0.4 ml **Buspirone** + 39.6 ml H_20 → *shaking* → 40 ml **Buspirone**
50mg/kg in EtOH 30% **5mg/kg in EtOH 0.3%**

All solutions were coded by people not involved in the research

Placebo (Control)
= same hydro-alchoolic solution
(0.3% EtOH)

Allopathic drug
= Buspirone or Diazepam
in the same hydroalchoolic solution
(0.3% EtOH)

22.10.2008

Schema per la codifica delle provette con i medicinali

Medicinale	Numero originale	Lettera CODIFICATA (a sorte tra A, B, C, D,E,F,G,H)
Gels 4CH:	N.1	.....
Gels 5CH:	N.2	.....
Gels 7CH:	N.3	.....
Gels 9CH:	N.4	.....
Gels 30CH:	N.5	.....
Placebo Non Dinamizzato:	N.6	.....
Placebo Non Dinamizzato:	N.7	.....
Buspirone 0.5 mg/ml	N.8	.....

I codici sono inseriti in una busta chiusa e sigillata che è consegnata in custodia a:

……

Firma (leggibile) di chi ha effettuato la codifica: ……

RANDOMIZATION of mice in the cages and rack

Albino CD1

Charles River Lab.

8 groups
(6 treatments
2 placebo control)

2 randomised
animals x cage

4 cages x group

Experiments approved
by ethical committee
No pain, no artificial stress

Open-Field ethological test

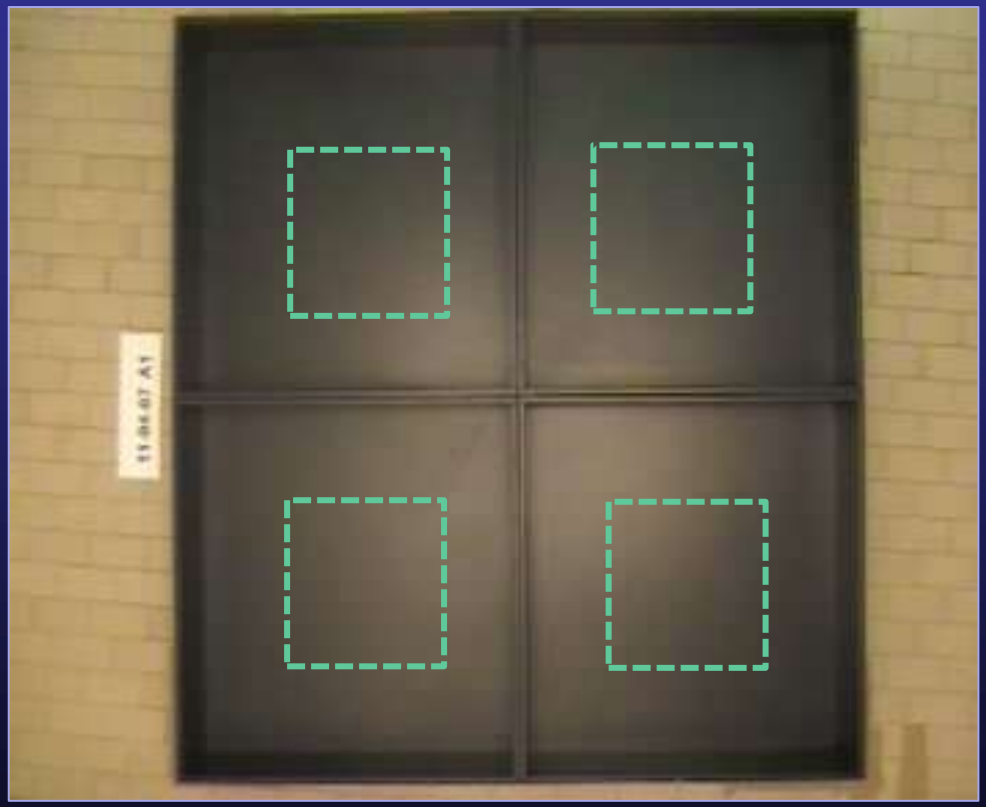
(anxiety-like response due to conflict between tendency to exploration and aversion to open space)

Main parameters:

→Total distance in 10 min. ("Locomotion")

→% Time in central area

→ Distance in centre



Experiments approved by ethical committee
No pain, no artificial stress

Light-Dark ethological test

(anxiety-like response due to conflict between tendency to exploration and aversion to light and to be alone in open space)

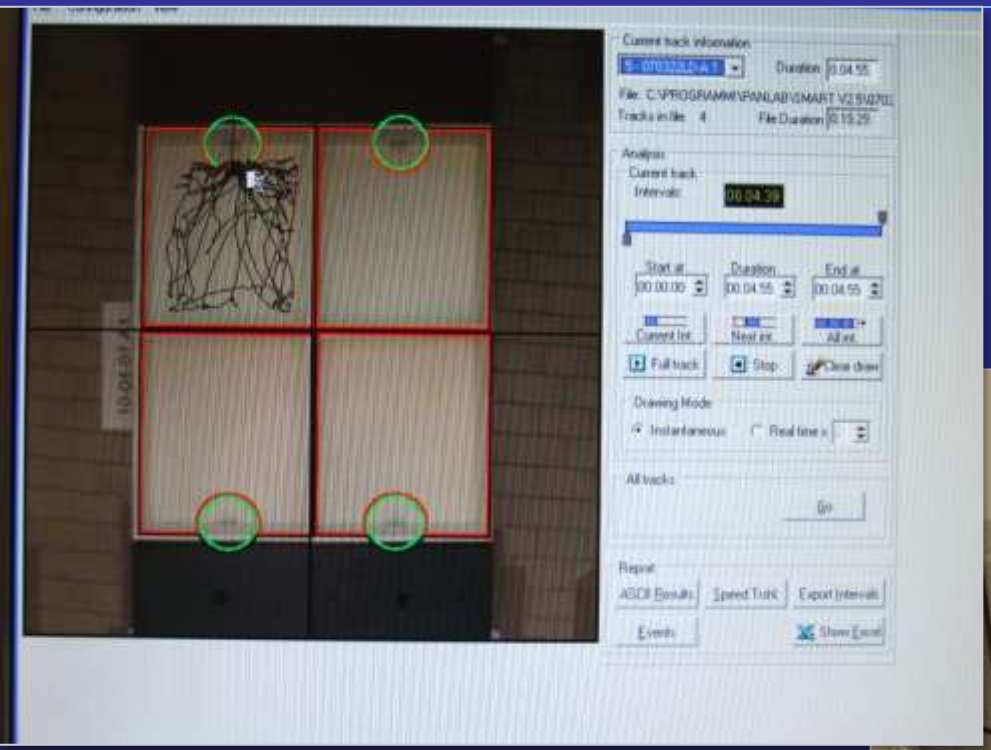
Main parameters:

→% Time in Light area

→ N. of transitions

Experiments approved
by ethical committee
No pain, no artificial stress

VIDEO-TRACKING AND AUTOMATIC CALCULATION OF BEHAVIOURAL SCORES



Tracking and Analysis with Smart software (Panlab Instruments)

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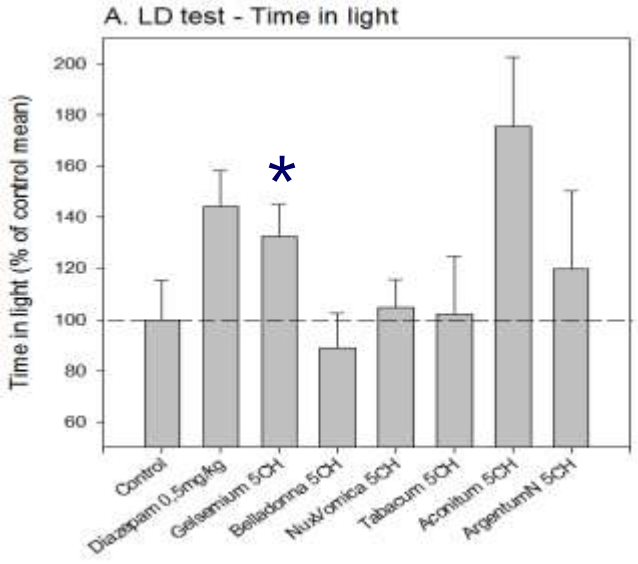
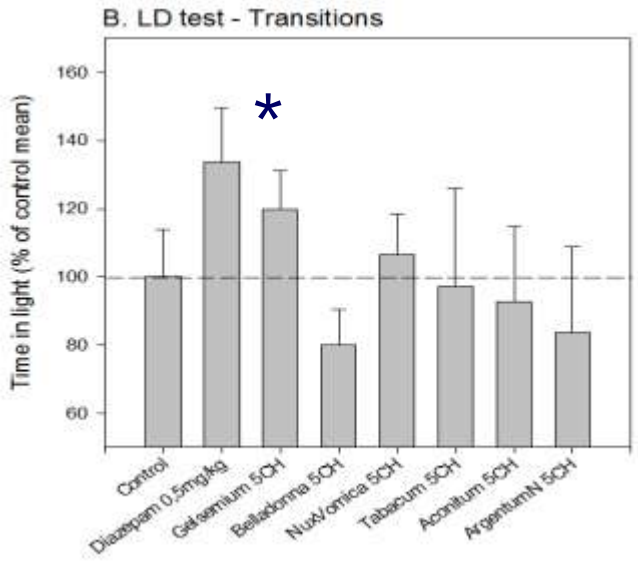
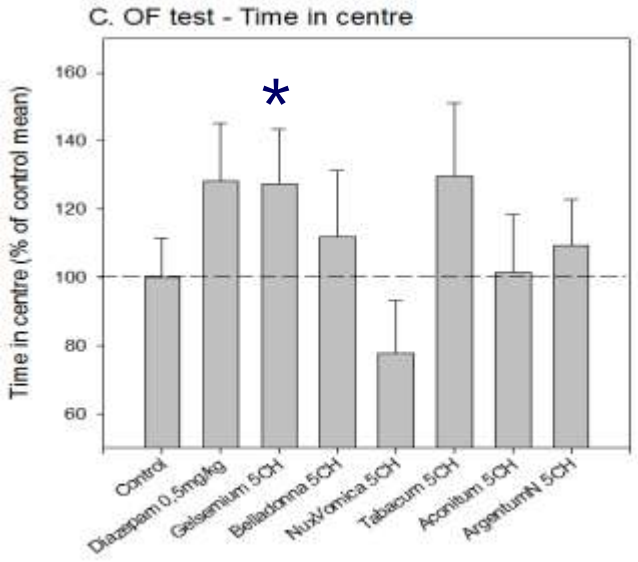
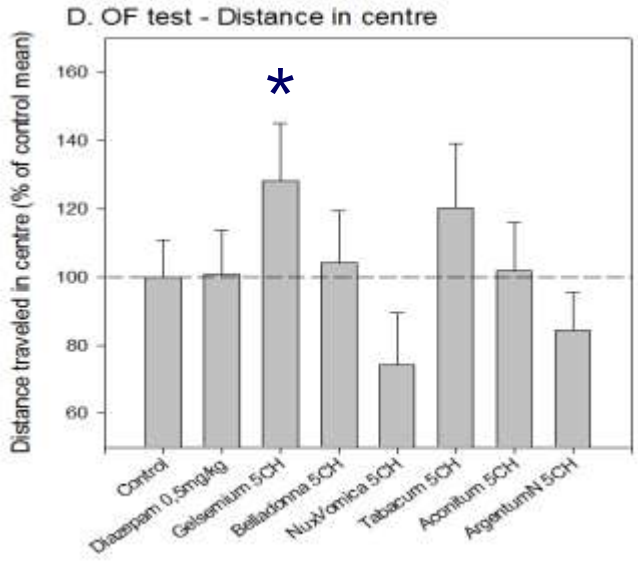
SCREENING TEST OF HOMEOPATHIC REMEDIES ON MICE BEHAVIOURAL MODELS (Liga Conference 2008)





*gelsemium.s

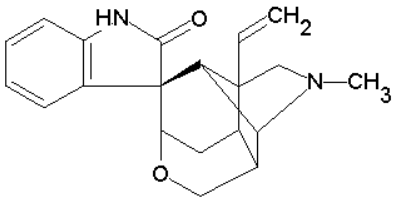




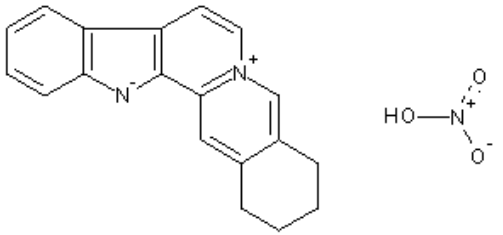
Gelsemium sempervirens


Gelsemium Sempervirens


CAROLINA-JESSAMINE
Gelsemium sempervirens (L.) *Ait. f.*
Logania Family



Gelsemine
Molecular Weight: 322,41)



Sempervirine
Molecular Weight: 335,36)

Gelsemium sempervirens

TRADITIONAL MATERIA MEDICA

Repertorial Materia Medica: Result of search by index in all repertories: [root:WALK] AND [root:AMEL]

- ✓**MIND - ANXIETY - walking - air, in open – amel. 7**
- ✓**MIND - ANXIETY - walking – amel. 8**
- ✓ **MIND - WALKING - air; in the open – amel. 20**
- ✓**GENERALS - WALKING - air; in open – amel. 135**
- ✓**GENERALS - WALKING - rapidly – amel. 19**
- ✓**GENERALS - WALKING - slowly – amel. 15**

Materia Medica (Boenninghausen, Murphy):

- ✓**MIND: FEELING AS IN DANGER OF FALLING**
- ✓**MIND: DREAD/DESIRE OF BEING ALONE**
- ✓**MIND: IMPATIENT AND IRRITABLE**
- ✓**MIND: NERVOUS DREAD OF APPEARING IN PUBLIC**

Drug analysis

Fax reçu de : 0472164223
BOURON
20 rue de la Libération
F - 69110 SAINTE-FOY-LES-LYON

15-05-08 09:42 Pg: 1
CERTIFICAT D'ANALYSE
N° LIMS : 62477

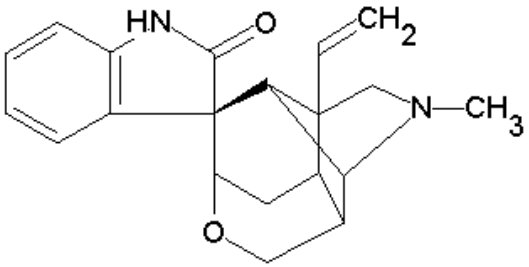
GELSEMIUM SEMPERVIRENS TM
G0B9.2TMGT1E

Date de fabrication	Quantité	N° de Lot
31 Mars 200	173.1 L	TH0082

Date de contrôle	N° de contrôle
26 Avril 20(4	C04046134

Partie Utilisée : Organes souterrains (1/10)
Description : Liquide jaune ambré, odeur aromatique.

ANALYSES	SPECIFICATIONS	RESULTATS
CARACTERES		
Couleur	conforme	conforme
IDENTIFICATION		
Chromatographie sur couche mince	conforme	conforme
ESSAI		
Teneur en éthanol	60 - 70 % V/V	63.7 % V/V
Méthanol	<0.05 %	<0.05 %
2-Propanol	<0.05 %	<0.05 %
Résidu sec	>0.50 %	1.37 %
DOSAGE		
Teneur en gelsémine	>0.010 %	0.021 %



Gelsemine
0.021% in MT

Gelsemium 9CH: 10^{-22} Mol/L ~ 1 molecule/mouse! (10,000,000,000,000,000 times less than in allopathic drug)

Gelsemium s. and mice behavioural responses (First series of studies: ECAM-J. 2009)

IMPACT FACTOR 2.53

eCAM Advance Access published September 14, 2009

eCAM 2009;Page 1 of 10
doi:10.1093/ecam/nep139

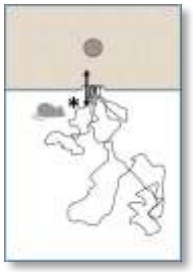
Original Article

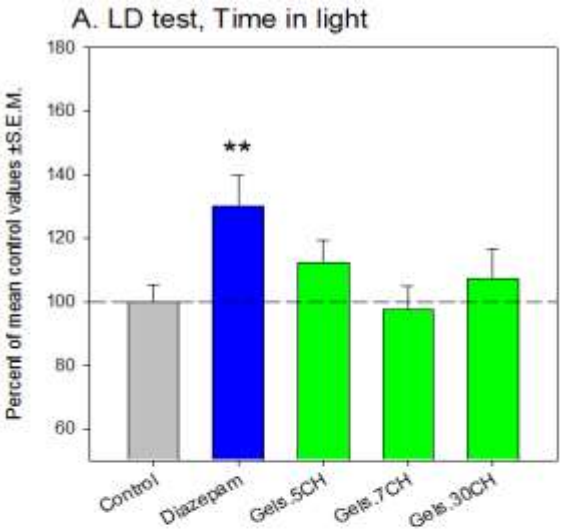
Homeopathic Doses of *Gelsemium sempervirens* Improve the Behavior of Mice in Response to Novel Environments

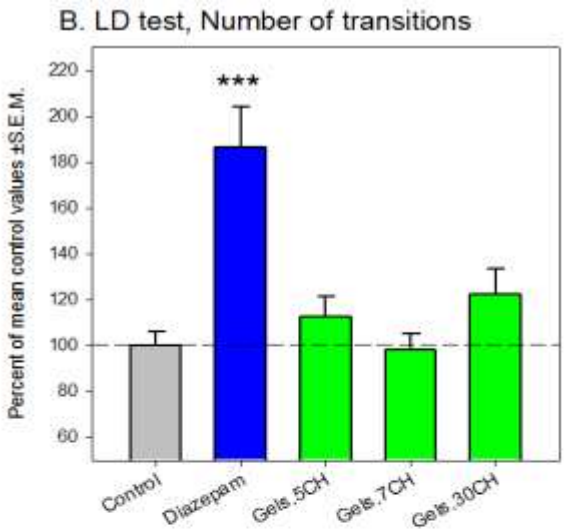
Paolo Bellavite[1], Paolo Magnani[1], Elisabetta Zanolin[2] and Anita Conforti[3]

[1]Department of Morphological Biomedical Sciences (Chemistry and Microscopy Section), [2]Department of Medicine and Public Health (Biomedical Statistics Section) and [3]Department of Medicine and Public Health (Medical Pharmacology Section), University of Verona, Verona, Italy

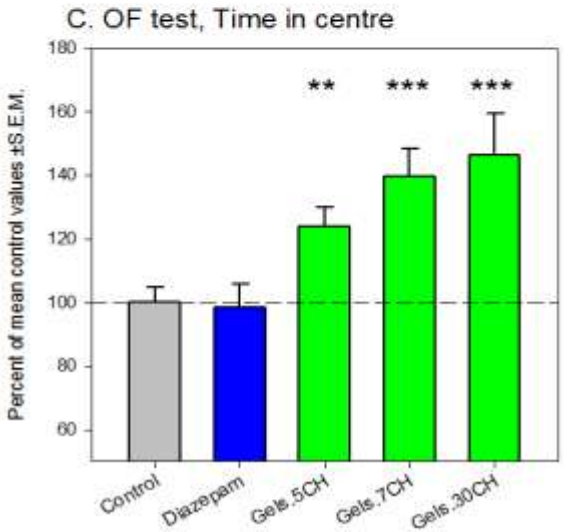
Gelsemium s. and mice behavioural responses (First series of studies: ECAM-J. 2009)

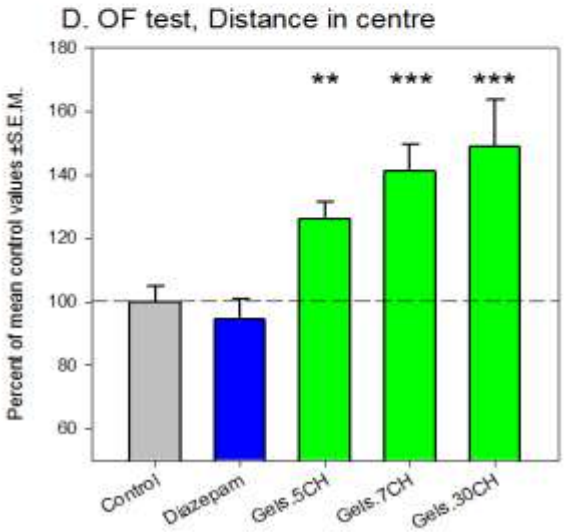






5CH= 8 exp
7CH= 3 exp
30CH= 2 exp
16 mice/exper





5CH= 8 exp
7CH= 3 exp
30CH= 2 exp
16 mice/exper

Scheme of the standard experiment

(2nd series with *Gelsemium s.*: dose-effect study)

- 8 groups of 8 animals (Harlan LAB), randomized 2 x cage
- 5 Dilutions of *Gelsemium*, 1 Buspirone and 2 Controls (placebo)
- All medicines/control coded by independent people

Albino CD1

Charles River Lab.

Start Housing Animal randomization

A	Medicine /Control1
B	Medicine/Control 2
C	Medicine/Control 3
D	Medicine/Control 4
E	Medicine/Control 5
F	Medicine/Control 6
G	Medicine/Control 7
H	Medicine/Control 8

- ✓**4CH**
- ✓**5CH**
- ✓**7CH**
- ✓**9CH**
- ✓**30C**
- ✓**Control (placebo)**
- ✓**Control (placebo)**
- ✓**Buspirone**

-7 0 1 2 3 4 5 6 7 8

days

Tracking Calculations (in blind conditions)

Open -Field Test

Light -Dark Test

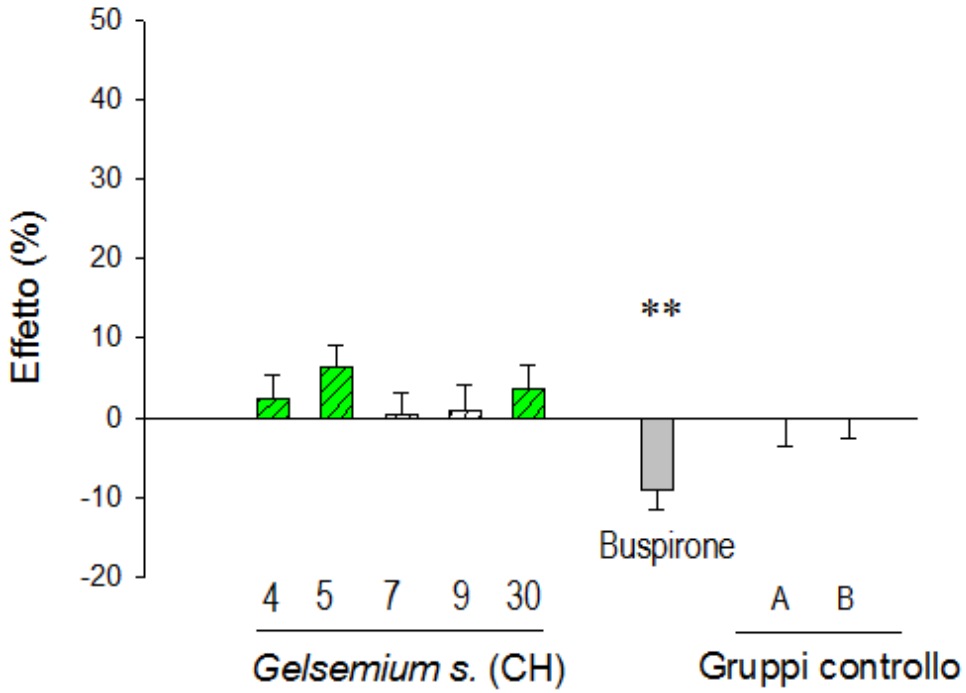
Experiments approved by ethical committee
No pain, no artificial stress

Behaviour of CD1 mice in the absence (control) and in the presence of *Gelsemium* or Buspirone (*Psychopharmacology* 2010)

Open-Field

Mean effect ± SEM of 6 experiments
total 48 mice/group

A: Open field:Total distance traveled (index of locomotion)



Global ANOVA for groups P=0.035

Behaviour of CD1 mice in the absence (control) and in the presence of *Gelsemium* or Buspirone (*Psychopharmacology* 2010)

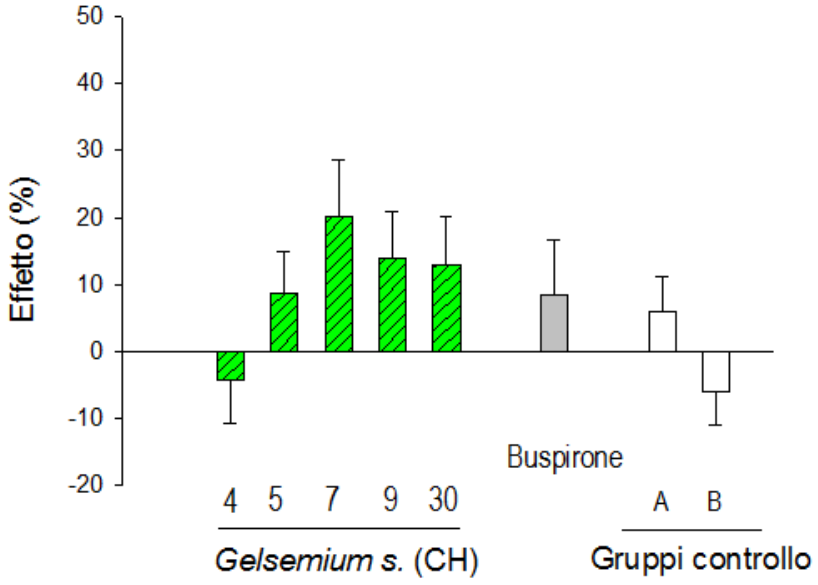
Open-Field

Mean effect ± SEM of 6 experiments
total 48 mice/group

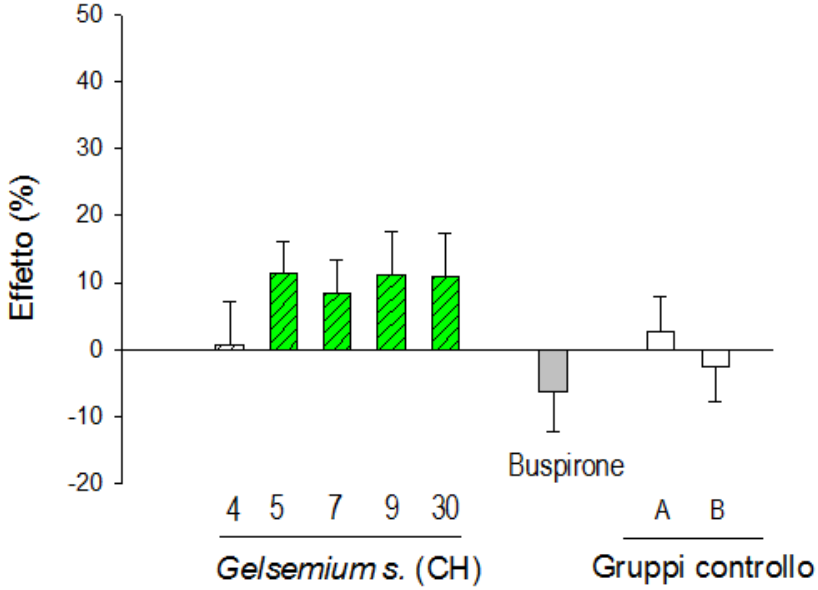
B: Global ANOVA for groups P=0.059

C: Global ANOVA for groups P=0.195

B: Open field: time spent in centre (anxyolitic-like effect)



C: Open field: distance traveled in centre



Behaviour of CD1 mice in the absence (control) and in the presence of *Gelsemium* or Buspirone (*Psychopharmacology* 2010)

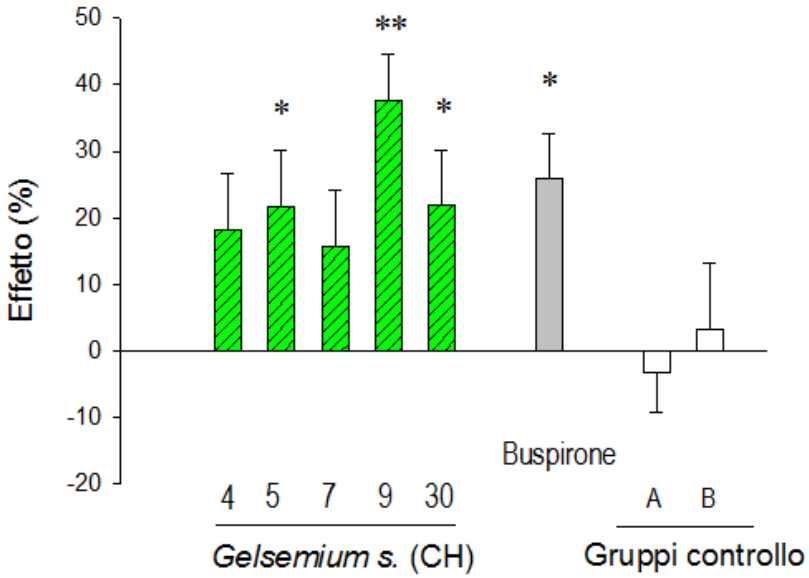
Light-Dark Mean effect ± SEM of 6 experiments
total 48 mice/group

D: Global ANOVA for groups P=0.0004

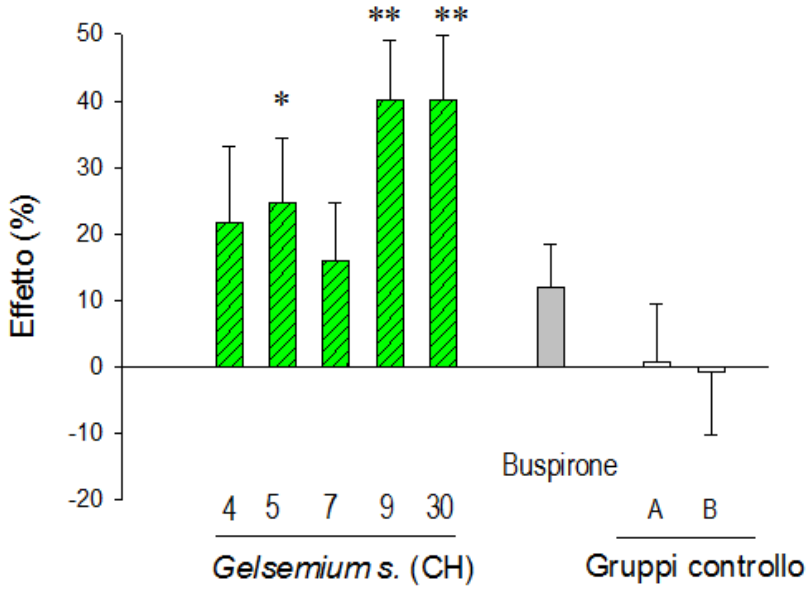
E: Global ANOVA for groups P=0.0002

P* <0,05
P<0,001**

D: Light-dark: time spent in light (anxyolitic-like effect)



E: Light-dark: number of transitions



Gelsemium s. and mice behavioural responses (Second series of studies: Psychopharmacology 2010)

Psychopharmacology (2010) 210:533–545
DOI 10.1007/s00213-010-1855-2

IMPACT FACTOR 3.65

ORIGINAL INVESTIGATION

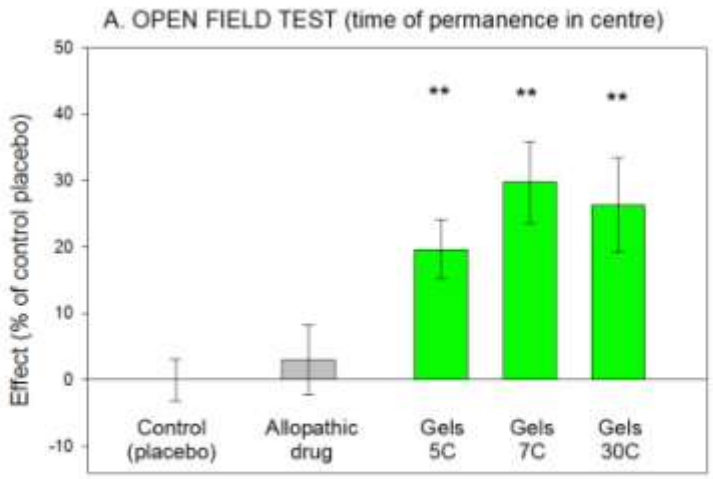
Dose-effect study of *Gelsemium sempervirens* in high dilutions on anxiety-related responses in mice

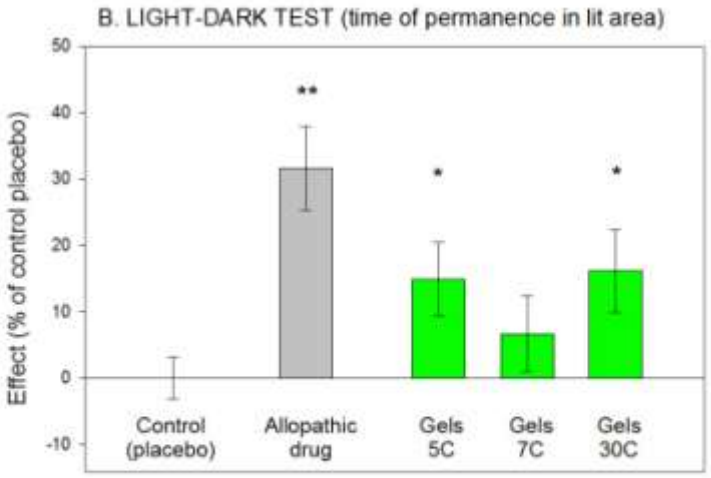
Paolo Magnani · Anita Conforti · Elisabetta Zanolin · Marta Marzotto · Paolo Bellavite

Received: 10 February 2010 / Accepted: 26 March 2010

Unpublished recent results of meta-analysis of two series where 5-7-30 CH were measured

Control-placebo: 14 exper
Allopathic drugs: 14 exper
Gels5CH: 14 exper
Gels 7CH: 9 exper
Gels 30CH: 8 exper

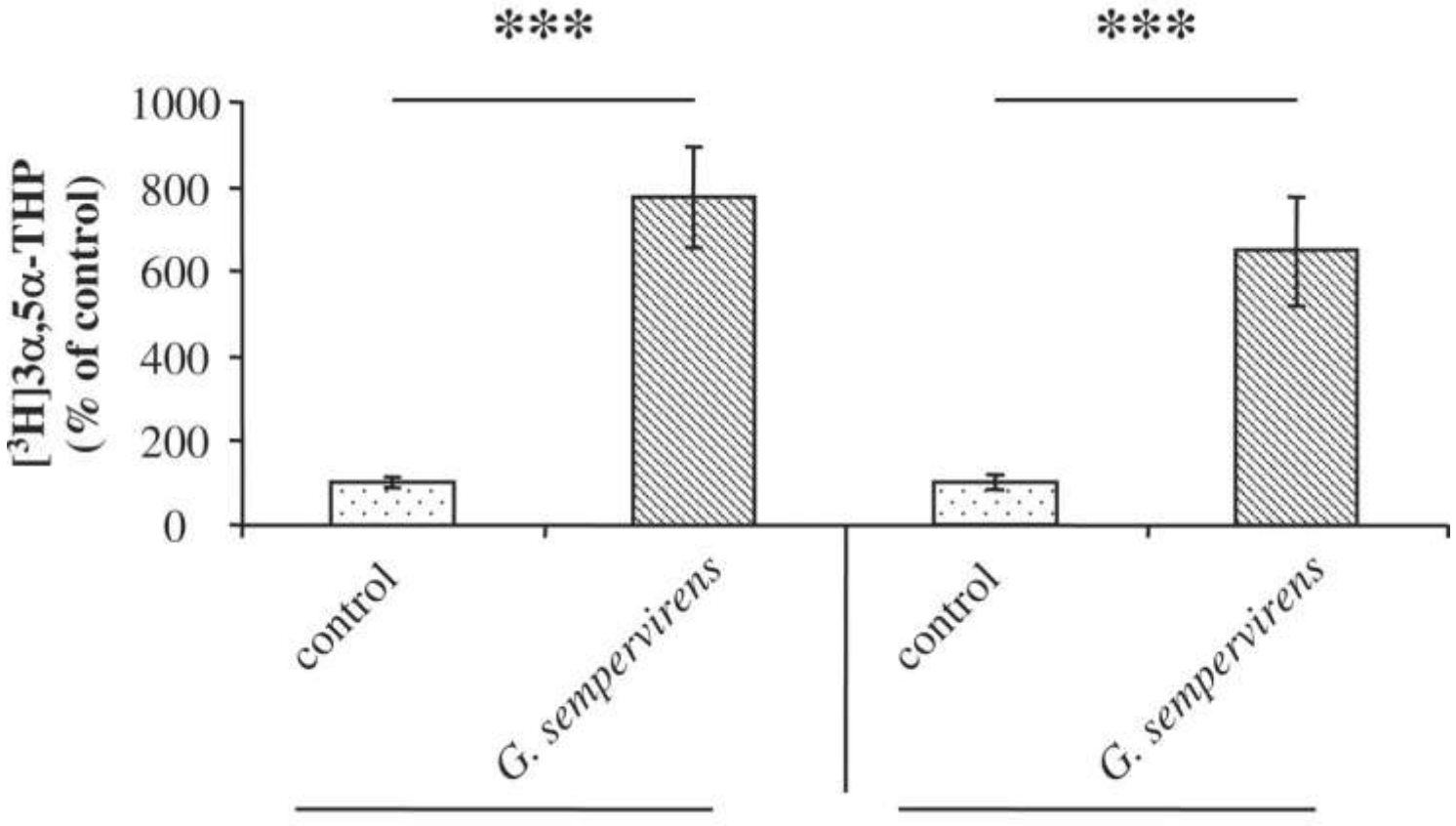




Gobal Anova for groups: p<0.01 in both tests
Post hoc (LSD): * <0.05, ** <0.01

Neurosteroid Allopregnanolone Formation in the Spinal Cord and Limbic System

Christine Venard et al., ECAM-J (advance access online)

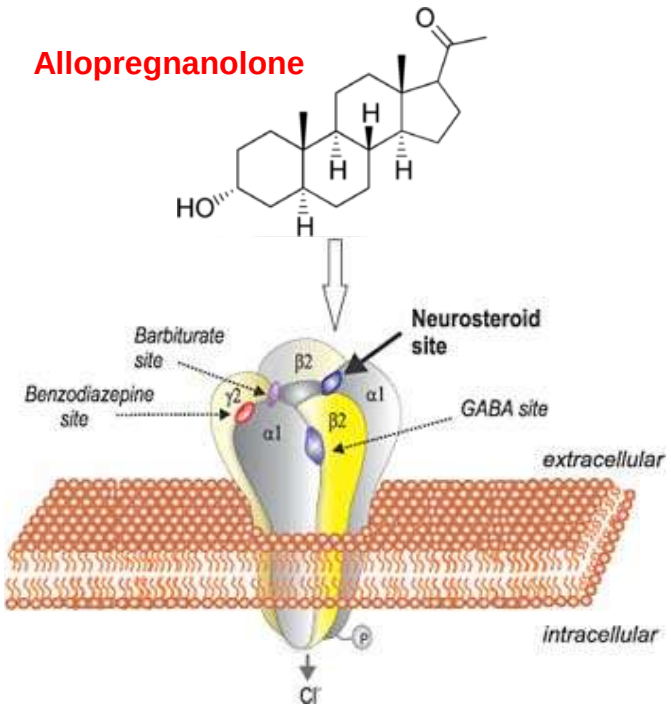


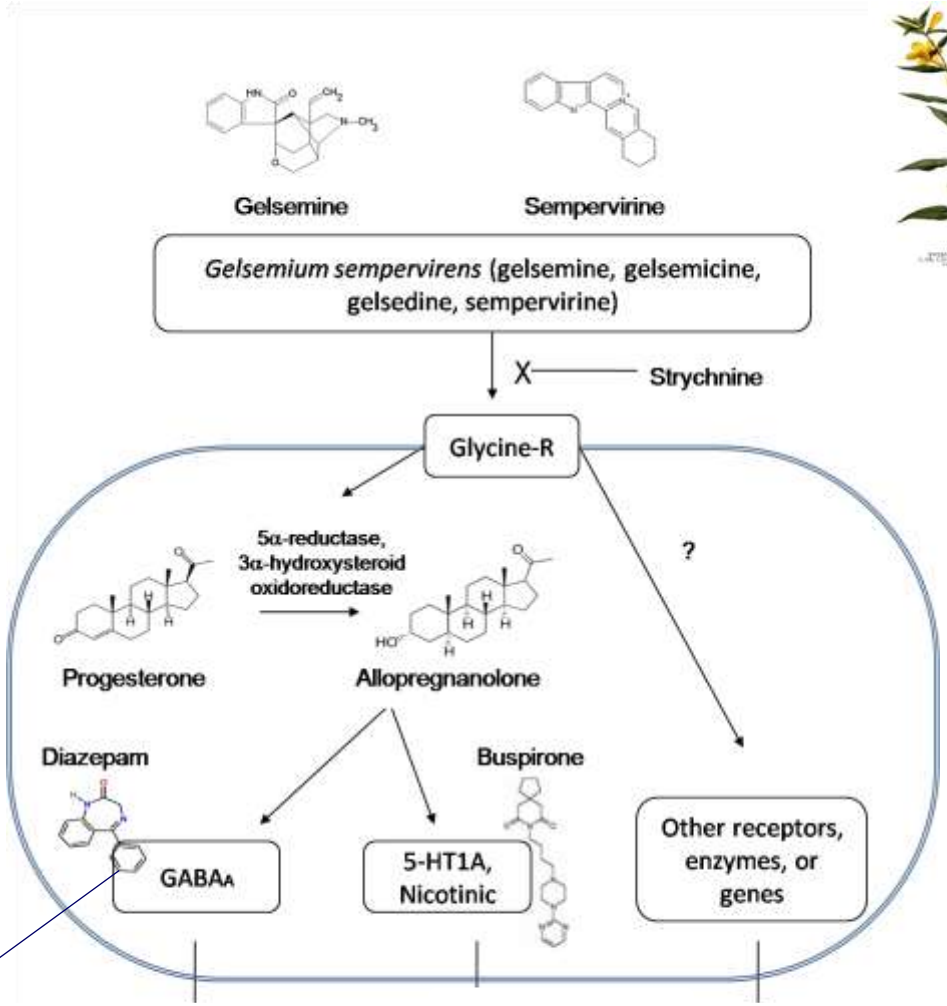
(≈10^{-22}mol/L)

(≈10^{-14} mol/L)

Working model of the mechanism of action of *Gelsemium sempervirens*

Allopregnanolone: an endogenous anxiolytic-like neurosteroid





The KEY-NOTES

Gelsemium sempervirens **improves some indexes of anxiety-like behavior significantly more than placebo in a large group of mice in rigorously blind conditions:**

- **The *Gelsemium* effects in mice concern a subset of symptoms which have been tested in our models:**
  - **aversion to open space**
  - **amelioration with movement**
  - **feeling in a danger**
  - **aversion to light**

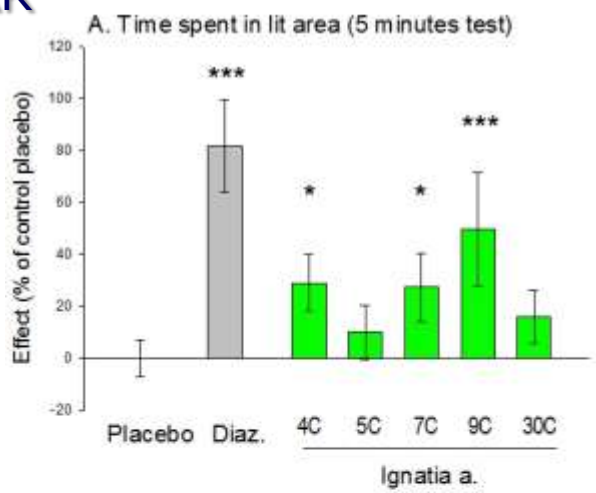
- **9CH and 30CH potencies appear to be more active than 4CH and 5CH**

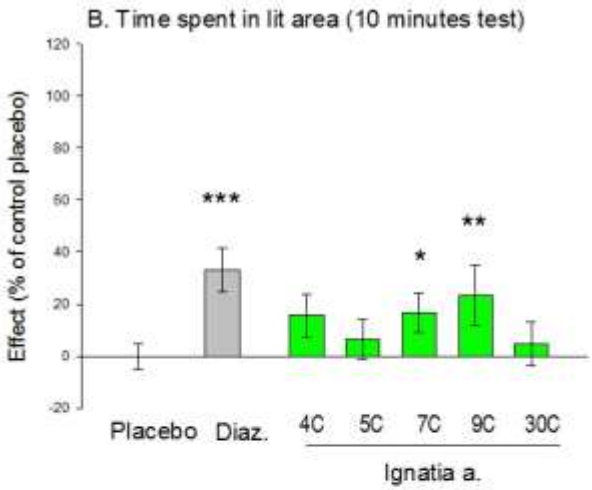
- **The anxiolytic-like effects of *Gelsemium* in Open Field are quite different from those of allopathic drugs (buspirone and diazepam)**

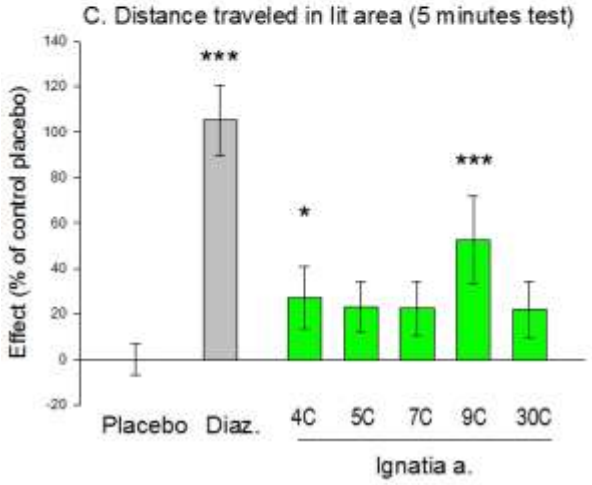
- ***Gelsemium* has no adverse effects on locomotion nor causes sedation (an effect shown by buspirone in chronic treatment)**

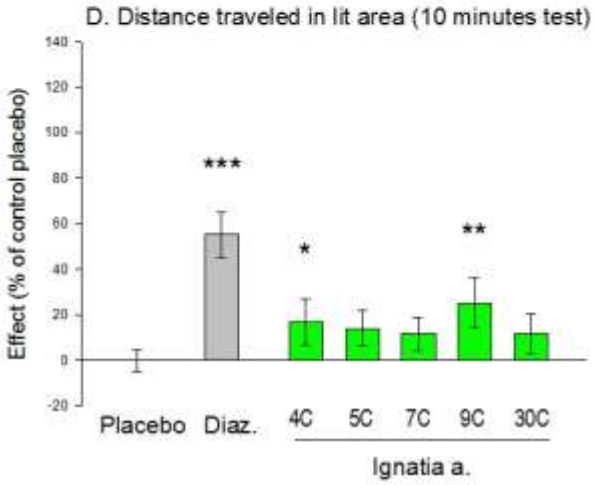
Unpublished recent results with Ignatia amara (5 experiments, 40 mice/group)

LIGHT-DARK TEST









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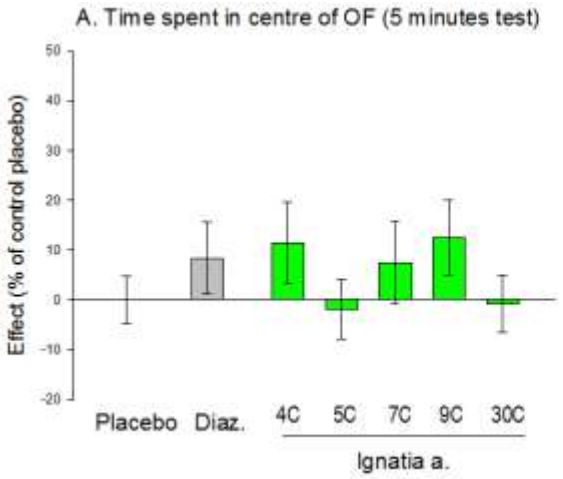
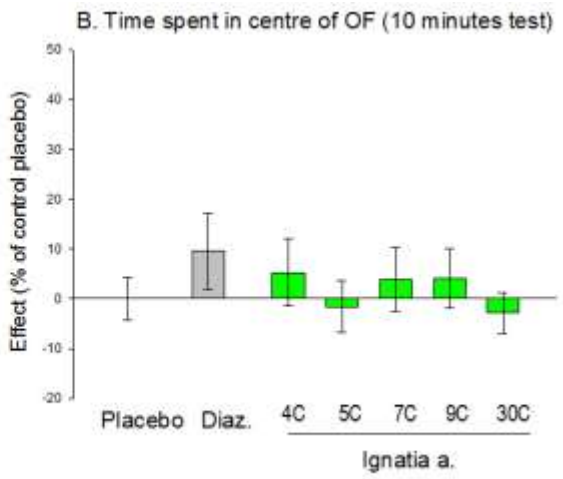
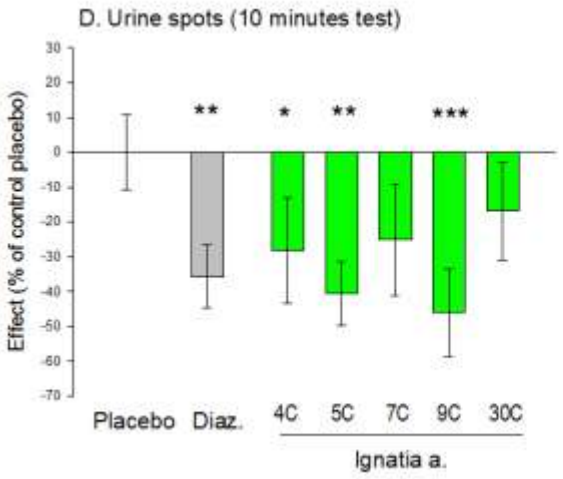
**=p<0.05
***=p<0.01

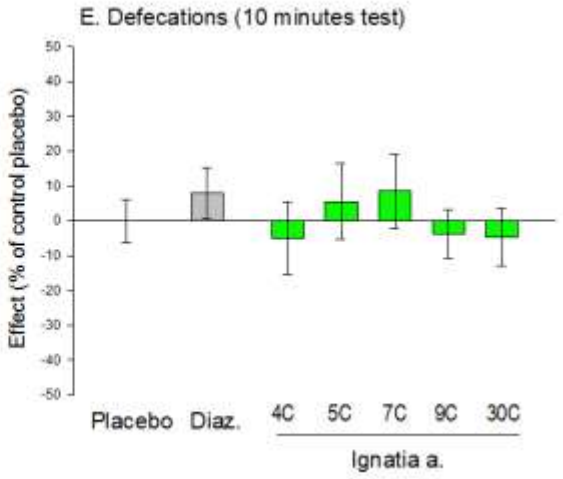
Unpublished recent results with Ignatia amara (5 experiments, 40 mice/group)

OPEN-FIELD TEST









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The Verona “Gelsemium” study group (2010)

We (and the mice) guarantee that HOMEOPATHY IS NOT A PLACEBO!

We thank
Boiron Laboratoires
Italian Research Ministry