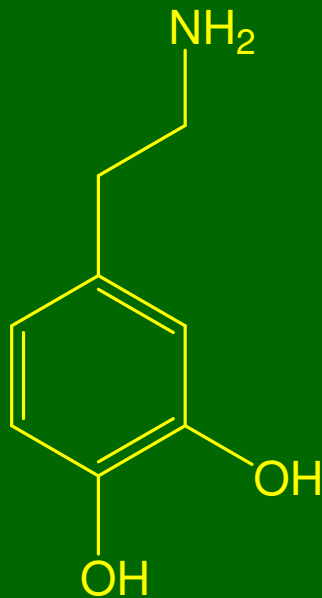


# **Not cathinone alone – dopamine, khat constituents and brain tissue**

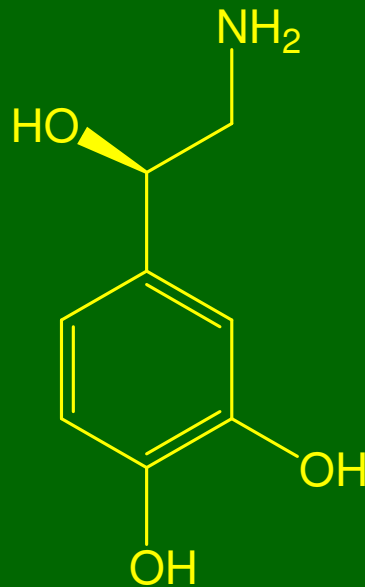
Peter Houghton, Muna Ismail  
and Sarah Salvage

*Pharmaceutical Sciences Research Division,  
Kings College London*

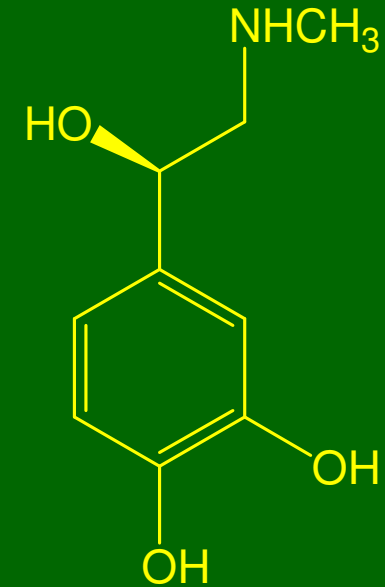
# Neurotransmitters



Dopamine



Noradrenaline

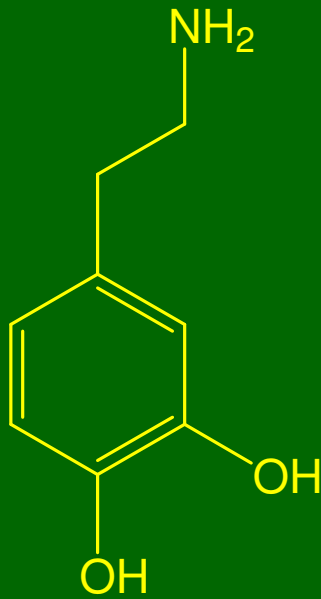


Adrenaline

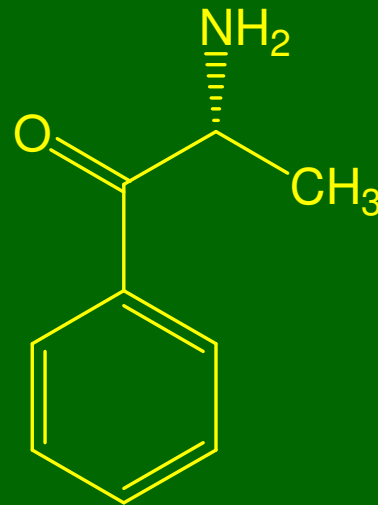
***Cause stimulation of sympathetic nervous system***

- *CNS excitation*
- *increased heart rate*
- *smooth muscle constriction in vascular system, intestine*

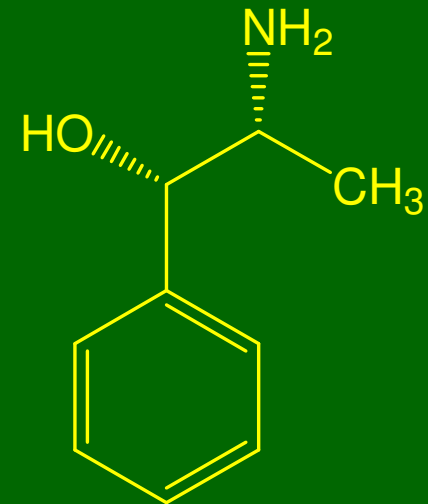
# Dopamine and phenylalkylamine khat constituents



Dopamine



Cathinone

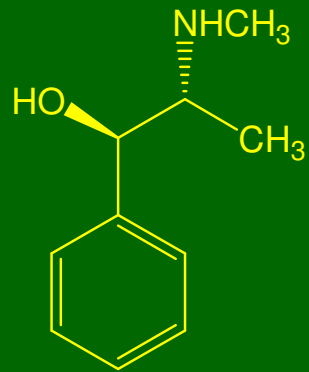


Cathine

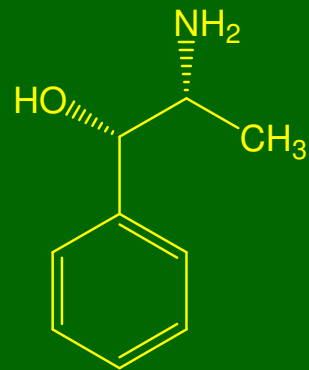
= (+) -norpseudoephedrine

Cathine isolated 1930, cathinone in 1980

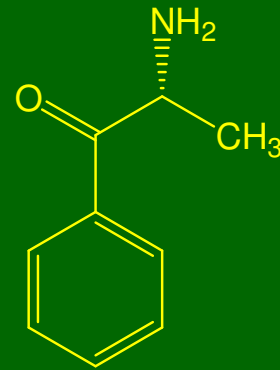
# Khat phenylpropylamines and sympathomimetic compounds



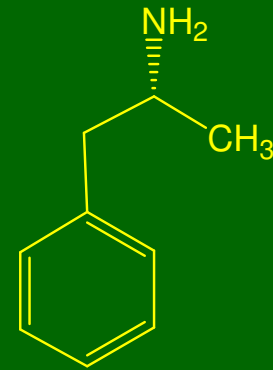
**(-)-Ephedrine**



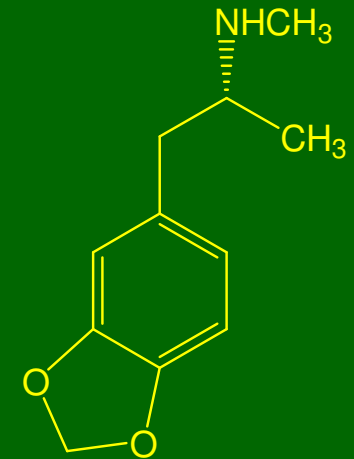
**Cathine**



**(-)- Cathinone**

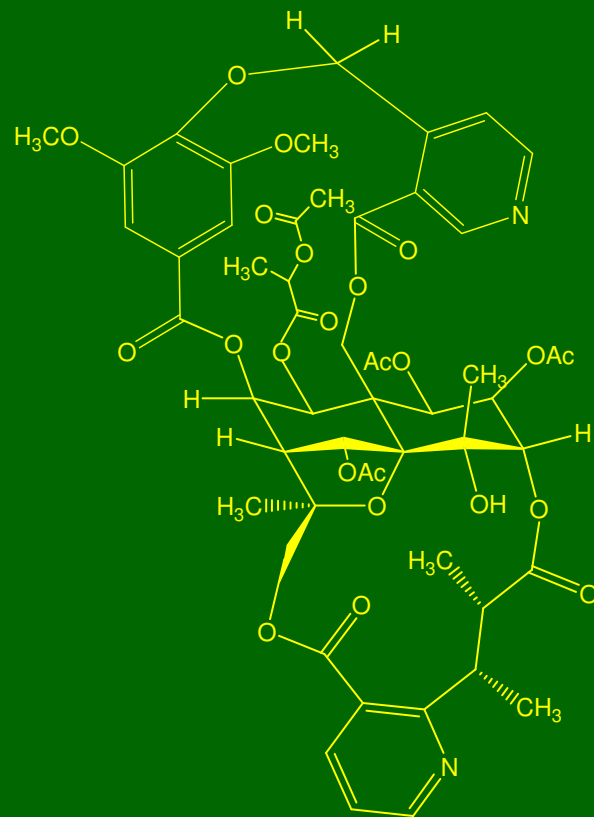


**(+)-Amphetamine**



**'Ecstasy'**

# Khat constituents - cathedulins



Cathedulin K11

First described in 1979  
Many different variations known  
Nothing known about biological activity

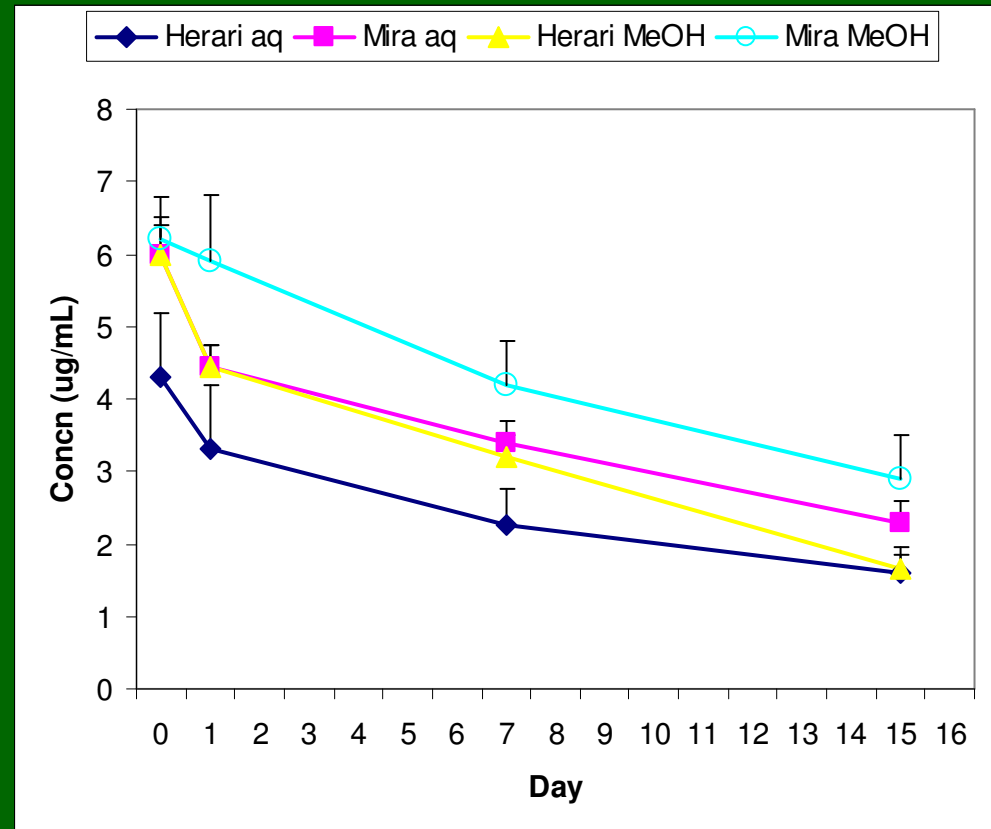
# Khat constituents – effect of storage

- Fresh leaves (less than 48 hours after picking) preferred
- Cathinone only found in young leaves, converted to cathine on storage
- Cathine main alkaloid in older leaves

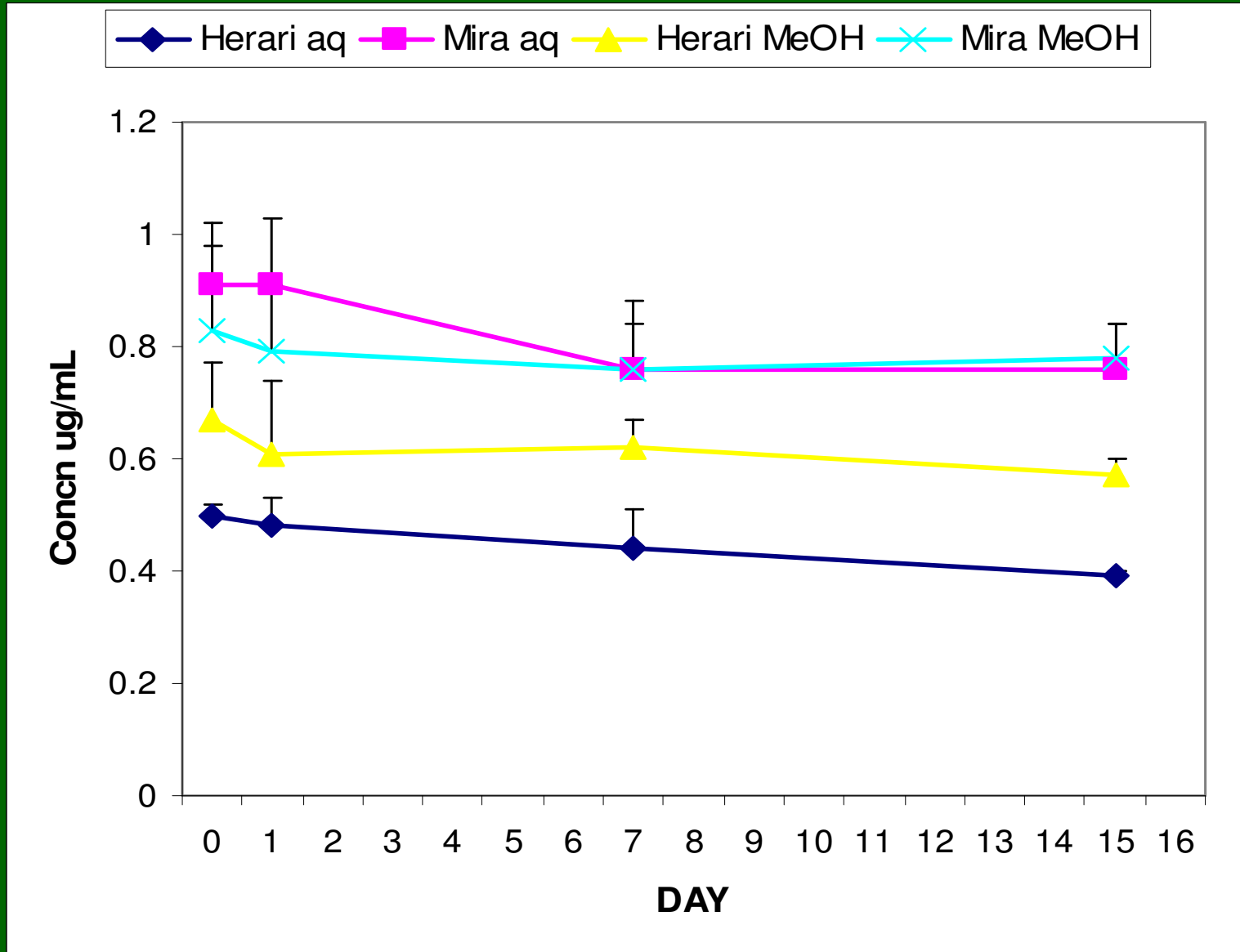
**What happens on storage?**

**Is all activity lost?**

# Cathinone levels in extracts over 15 days storage



# Cathedulin K11 levels in extracts over 15 days storage





# Khat constituents – effect of storage

- Cathinone degrades more quickly than cathedulins
- Cathedulins likely to be present in older leaves and stored material

**What (if any) activity remains due to cathedulins?**

**Are there differences between Mira (Kenya) and Herari (Ethiopia) material?**

# Khat constituents variation

HPLC chromatogram of aqueous extract

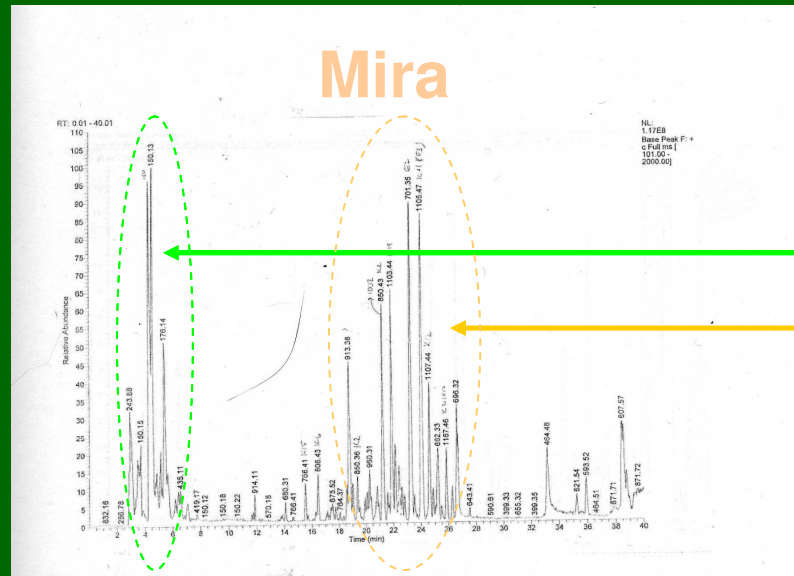


Figure2-22 LC/MS profile of fresh fMA extract

Phenylalkylamines  
Cathedulins

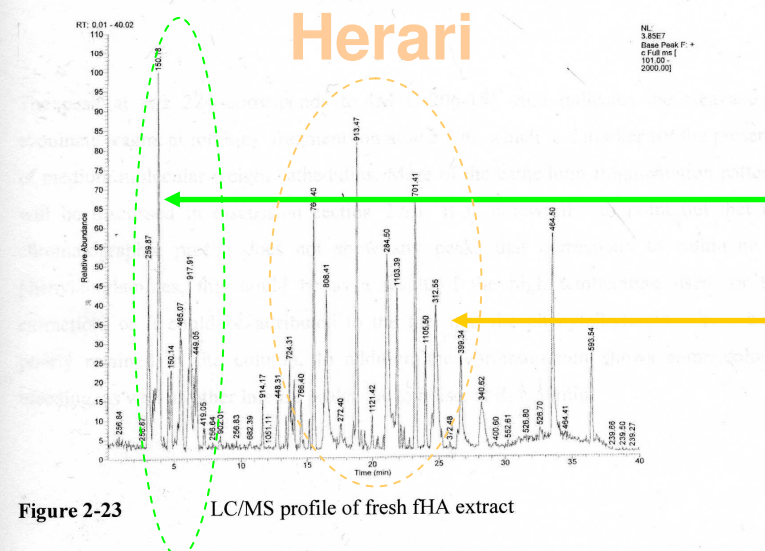


Figure 2-23 LC/MS profile of fresh fHA extract

Phenylalkylamines  
Cathedulins

# Khat constituents variation

Cathinone, norephedrine and cathedulin K11  
quantified ( $\mu\text{g/mL}$ )<sup>\*</sup> in extracts using HPLC

Compound	Mira methanol	Mira aqueous	Herari methanol	Herari aqueous
(-)-Cathinone	4.33 $\pm$ 1.56	3.72 $\pm$ 0.69	4.65 $\pm$ 2.00	2.90 $\pm$ 1.21
(-)-Norephedrine	18.07 $\pm$ 3.04	16.43 $\pm$ 3.03	19.15 $\pm$ 4.40	15.20 $\pm$ 2.03
Cathedulin K11	0.60 $\pm$ 0.15	0.63 $\pm$ 0.24	0.57 $\pm$ 0.12	0.48 $\pm$ 0.10

\* Mean  $\pm$  SEM    n = 5

# Khat constituents variation

- *Mira and Herari varieties have different cathedulin profiles*
- *Some variation in phenylalkylamine composition: Herari has slightly higher amounts of cathinone*
- *Levels of cathedulins (expressed as cathedulin K11) very similar*
- *Any difference in activity may be related to the cathedulins*

# Khat constituents and dopamine

- Excitatory effect of phenylalkylamines due to release of dopamine (DA) in brain
- Depletion of DA in striatum nigrum in brain is a feature of Parkinsonism
  
- Do khat constituents affect DA release?
- Do khat constituents bind to DA receptors in brain?

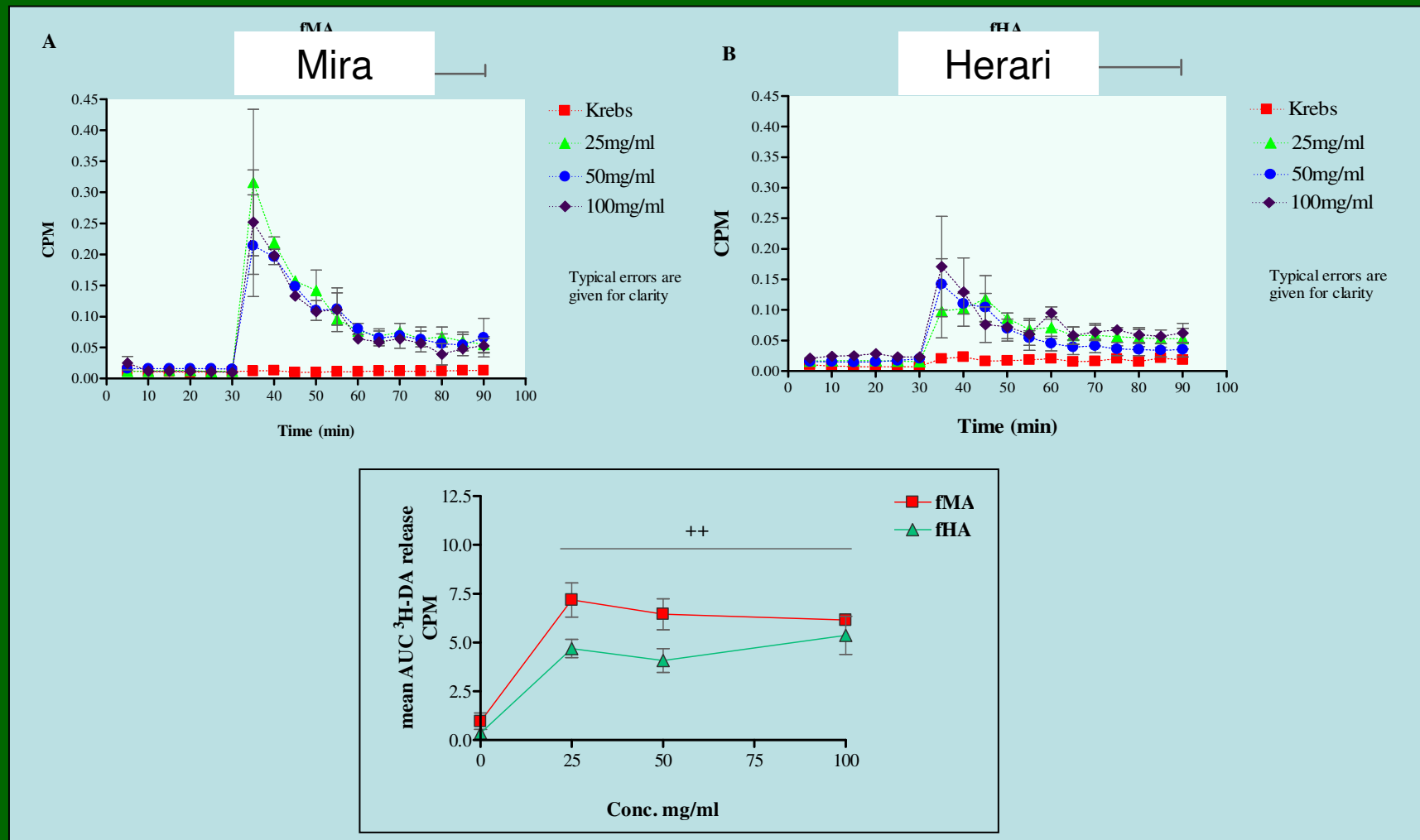
# Khat constituents and dopamine

- Aqueous and methanolic extracts made from Mira material
- Polyphenols removed by solid phase extraction using C18 silica
- Extract put through flash column using reverse-phase chromatography
- Fractions monitored by HPLC
- Fractions showing cathedulins combined
- Pure cathinone and norephedrine used

# Measuring DA release

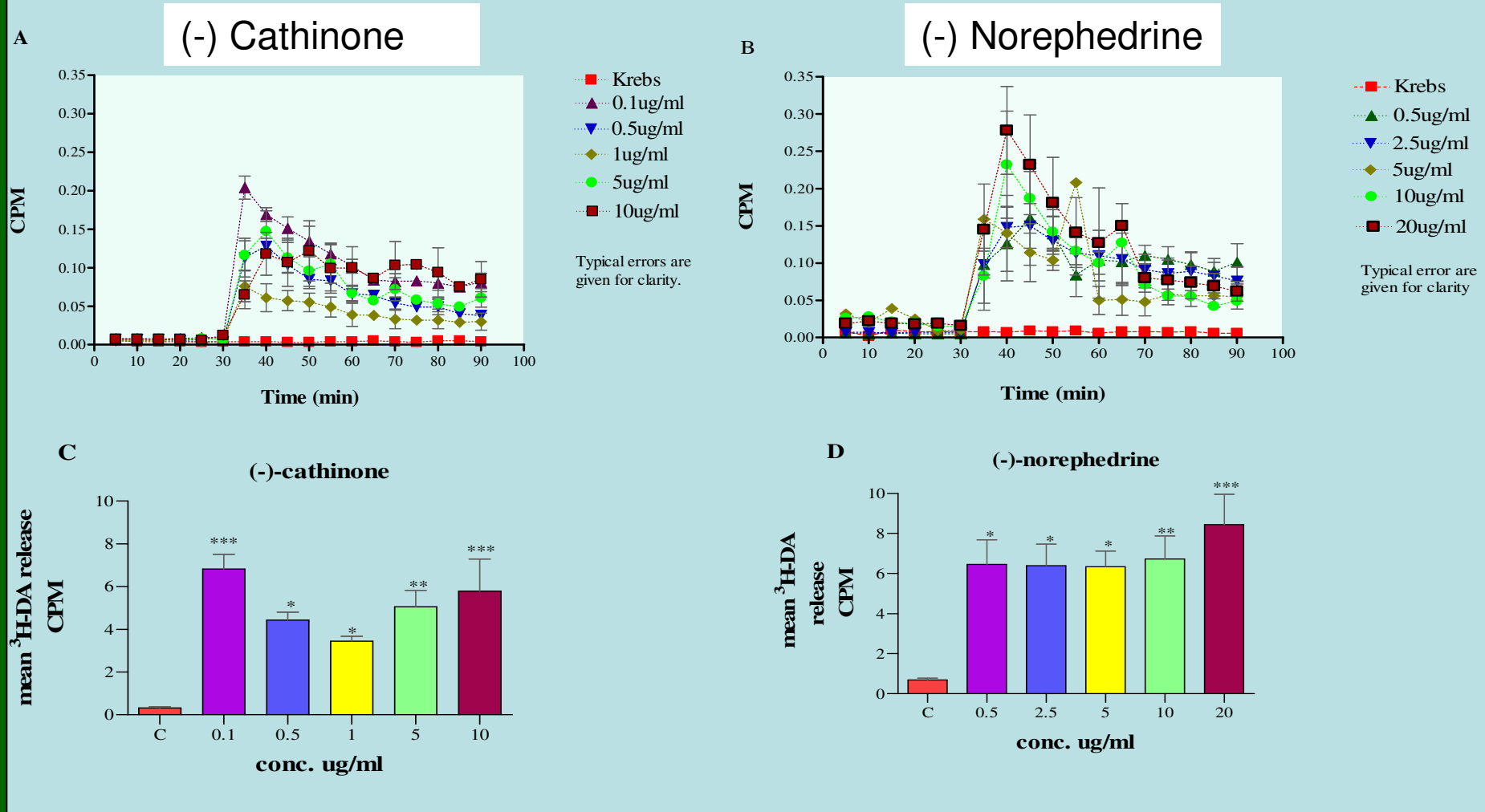
- Corpus striatum removed from brains of killed rats
- Slices made - 300 $\mu$ m thick
- Tissue incubated in  $^3\text{H}$  dopamine ( $^3\text{H}$  DA) – some incorporated into cells
- Washed with Krebs solution- removes excess DA
- Slices put in superfusion chambers
- Perfused with solution of substance under test for 100 min
- Activity of perfusate: counts per min (cpm) measured by scintillation counting at 5 min intervals
- Cpm plotted against time and area under curve measured as an indicator of DA release

# Dopamine (DA) release from striatal slices over 100 mins from aqueous extracts

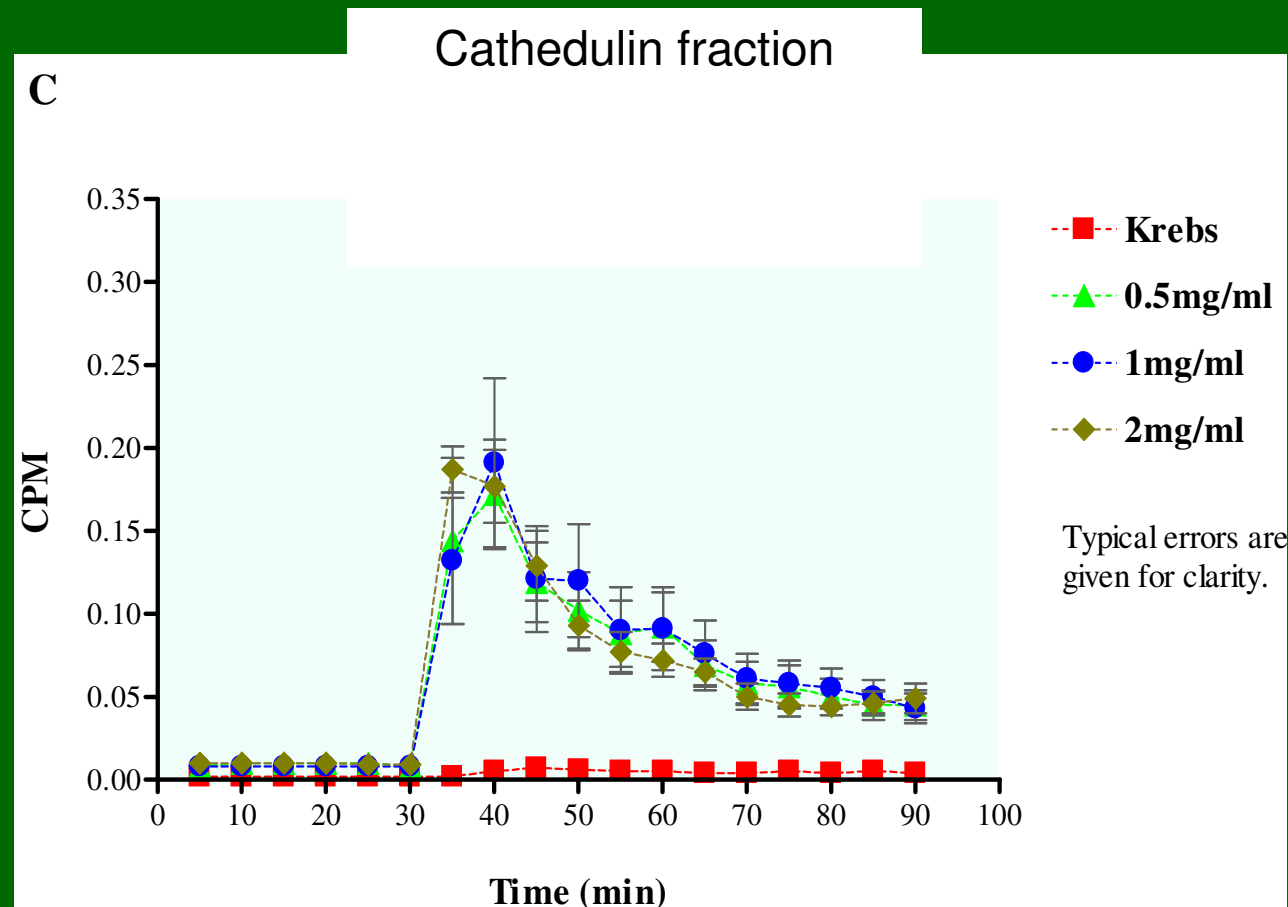




# Dopamine (DA) release from striatal slices over 100 mins from compounds



# Dopamine (DA) release from striatal slices over 100 mins from cathedulin fraction



## Dopamine (DA) release from striatal slices

### CONCLUSIONS

- Cathinone and norephedrine cause release of DA from tissue slices (previously known)
- Norephedrine dose-related response
- Cathinone gives a U-shaped response
- Extracts and cathedulin fractions cause release, but not dose-related
- Mira extract gives slightly greater DA release (*HPLC showed higher levels of alkaloids in Mira aq ext cf. Herari aq ext*)

**THIS IS THE FIRST REPORT OF ANY BIOLOGICAL EFFECT DUE TO CATHEDULINS**

# Measuring binding to DA receptors

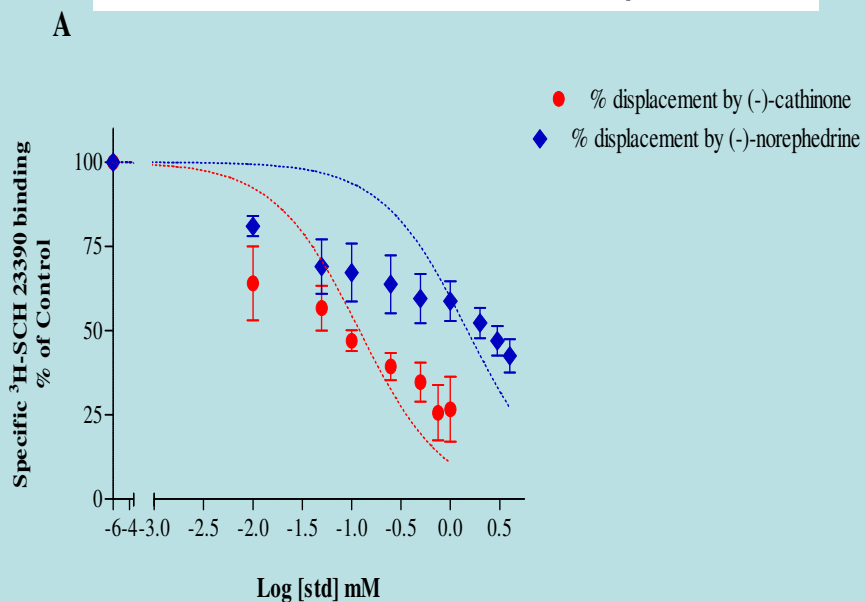
- Uses measurement of displacement of radiolabelled selective binder to receptor
- Two types of DA receptor D1 and D2
- D1 – basis for many psychomotor effects, cognitive function, cardiovascular function. **Selective binder is SCH 23390**
- D2 – Implicated in several neurological and psychiatric disorders and in reward-seeking behaviour associated with substance misuse. (decreased DA 2 receptors with longterm psychostimulant use). **Selective binder is Spiperone**

# Measuring binding to DA receptors

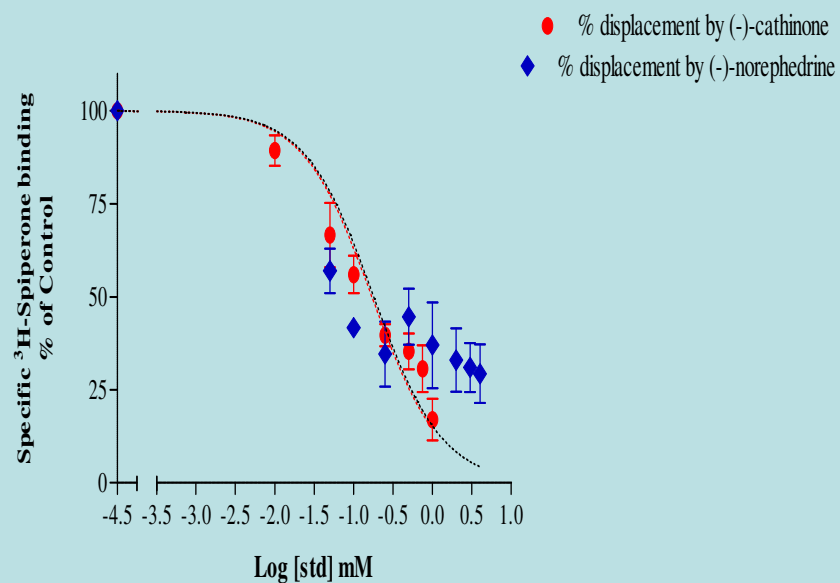
- Corpus striatum removed from brains of killed rats
- Striata homogenised in TRIS buffer
- Washed several times and centrifuged to remove supernatant
- Pellet re-suspended in buffer and range of concentrations of test substance and constant dose of known activity  $^3\text{H}$ -labelled selective binder added
- Incubated at  $37^\circ$  for 20 min, filtered under vacuum
- Unbound ligand removed by washing with buffer
- Filter paper placed in scintillation fluid and cpm measured as indicator of radioactivity
- Specific binding calculated
- Plotted against concn of test substance

# Displacement of selective receptor binding compounds by cathinone and norephedrine

$^3\text{H}$ -SCH23390 D1 receptor



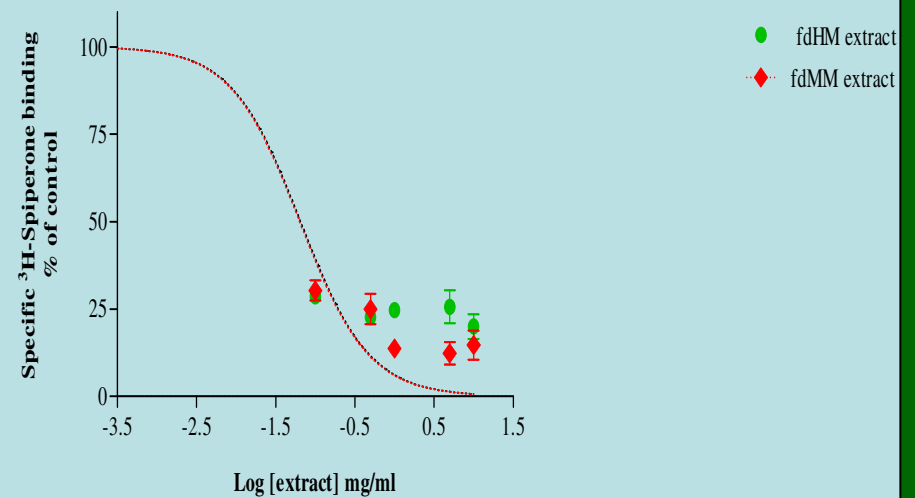
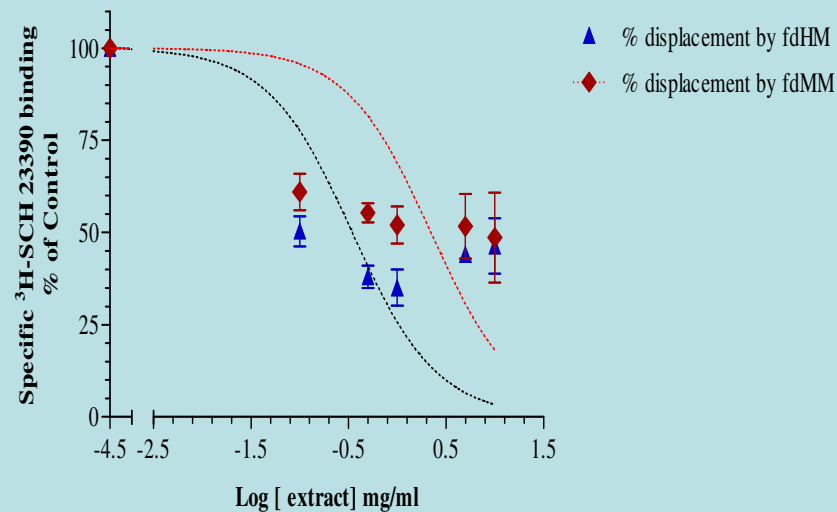
$^3\text{H}$ -Spiperone D2 receptor



# Displacement of selective receptor binding compounds by methanol extracts of Mira and Herari khat

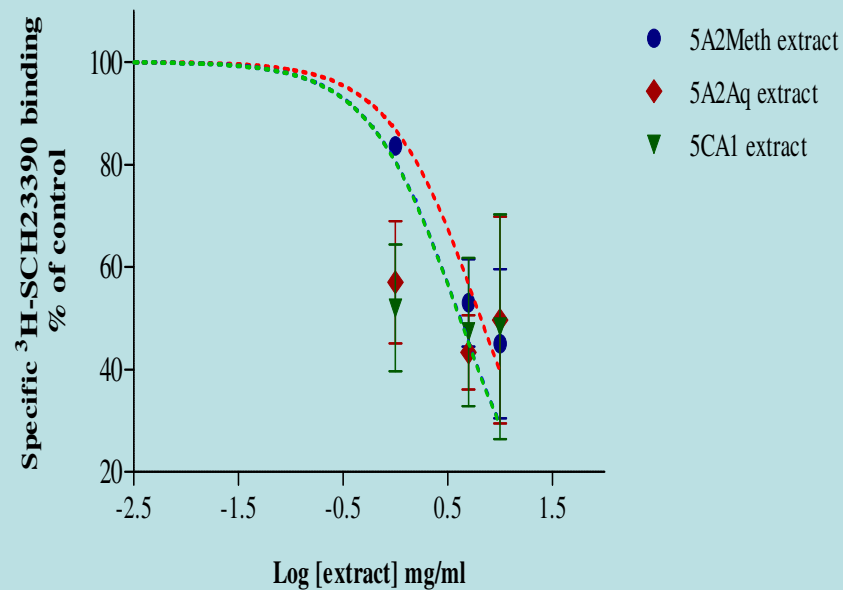
$^3\text{H}$ -SCH23390 D1 receptor

$^3\text{H}$ -Spiperone D2 receptor

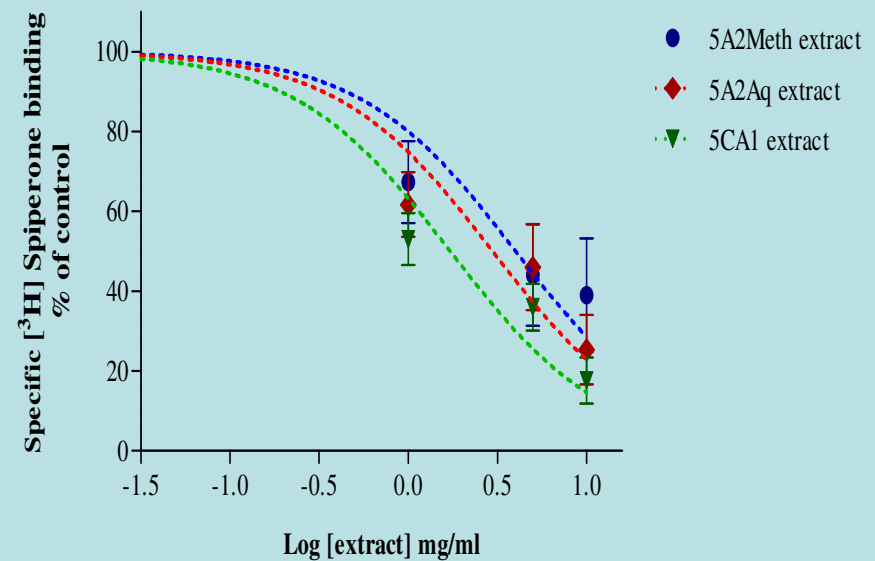


# Displacement of selective receptor binding compounds by cathedulin fractions

$^3\text{H-SCH23390}$  D1 receptor



$^3\text{H-Spiperone}$  D2 receptor





## Binding to DA receptors

# CONCLUSIONS

- Cathinone has a greater binding effect than norephedrine on D1 receptors
- Cathinone and norephedrine have similar binding effects on D2 receptors
- Extracts have weak, non dose-related binding effects on D2 receptors ; the binding to D1 receptors is stronger but not really dose-related
- Cathedulins bind to both receptors, possibly more strongly to D2 type

# CONCLUSIONS REGARDING ACTIVITY AND USE

**NB Very difficult to extrapolate in vitro results to in vivo/clinical situation!**

- Mira variety of khat has slightly higher levels of compounds and activity – probably not significant
- Cathedulins affect dopamine release and have dopaminergic action – older samples may still have some effect
- Cathedulins appear to bind to D2 receptors – possible effect on reward-seeking, and therefore ‘addiction’???

# CONSTITUENTS of KHAT and DOPAMINE-RELATED EFFECTS IN CNS

- Cathinone and related compounds not the only actives
- Cathedulins appear to make some contribution
- Animal and clinical studies needed
- Volatile oils and tannins present not investigated
- Possible study to link khat use with incidence/alleviation of Parkinsonism?

**MORE WORK NEEDED!**