Not cathinone alone – dopamine, khat constituents and brain tissue

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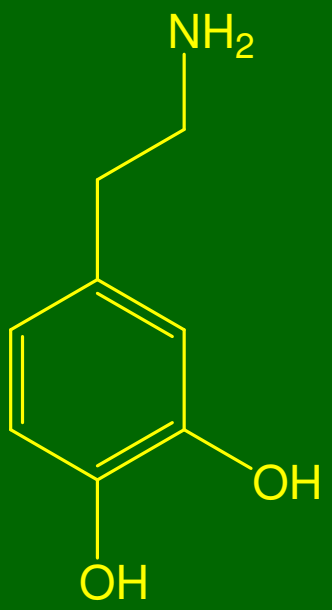
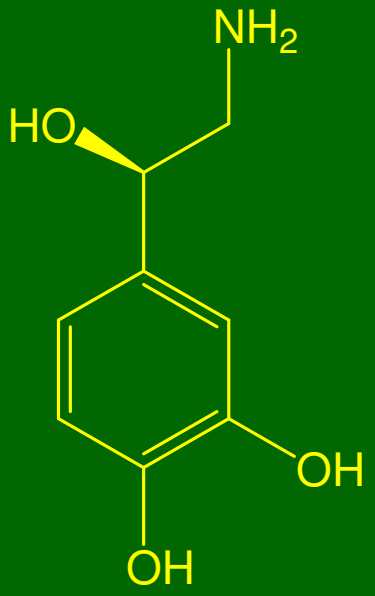
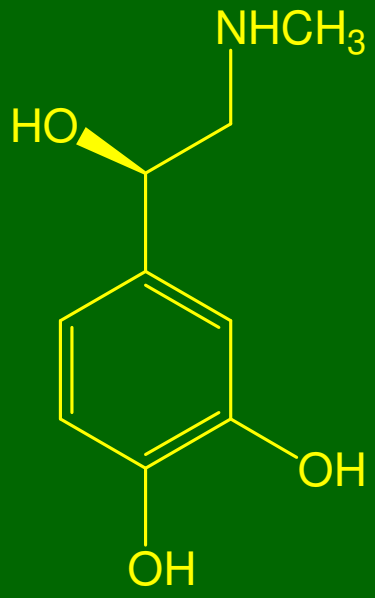
Neurotransmitters



Dopamine



Noradrenaline



Adrenaline

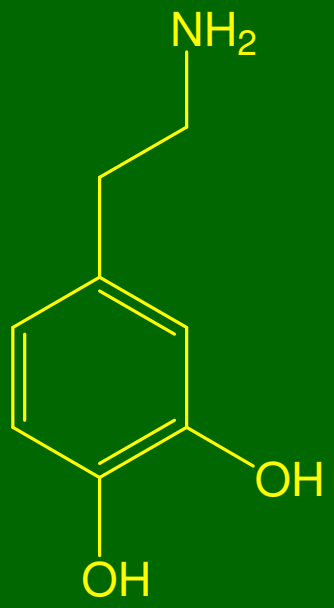
Cause stimulation of sympathetic nervous system

- CNS excitation

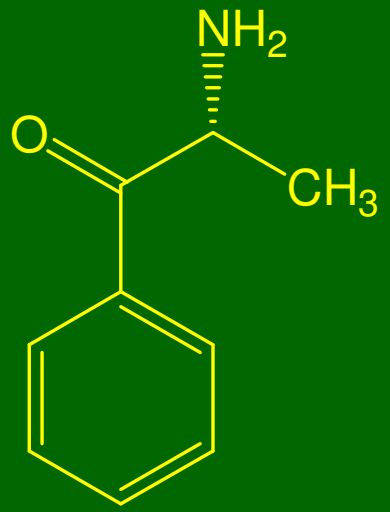
- increased heart rate

- smooth muscle constriction in vascular system, intestine

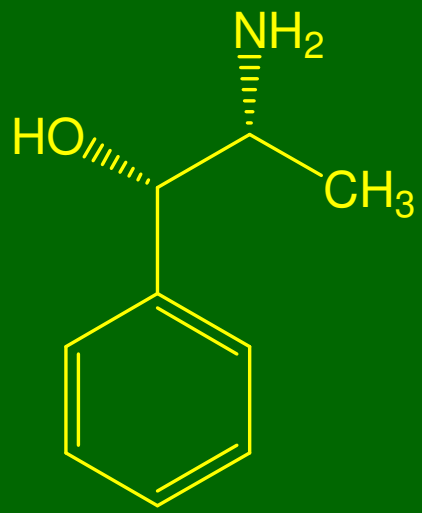
Dopamine and phenylalkylamine khat constituents



Dopamine



Cathinone

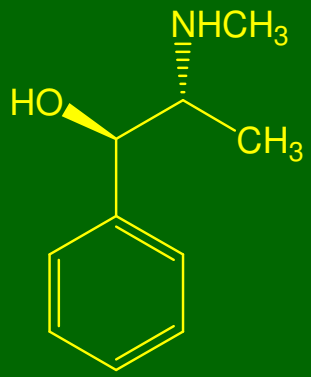


Cathine

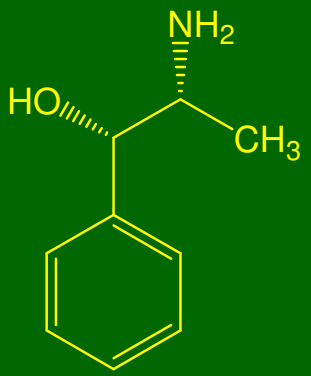
= (+) -norpseudoephedrine

Cathine isolated 1930, cathinone in 1980

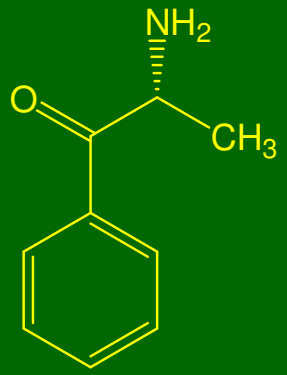
Khat phenylpropylamines and sympathomimetic compounds



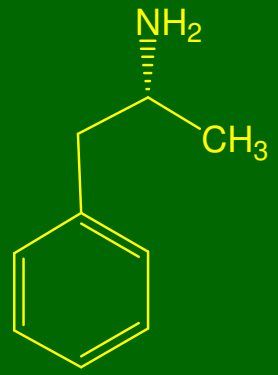
(-)-Ephedrine



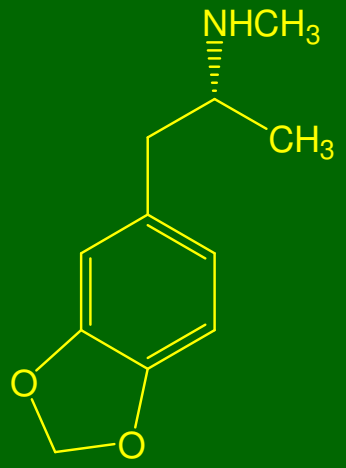
Cathine



(-)- Cathinone

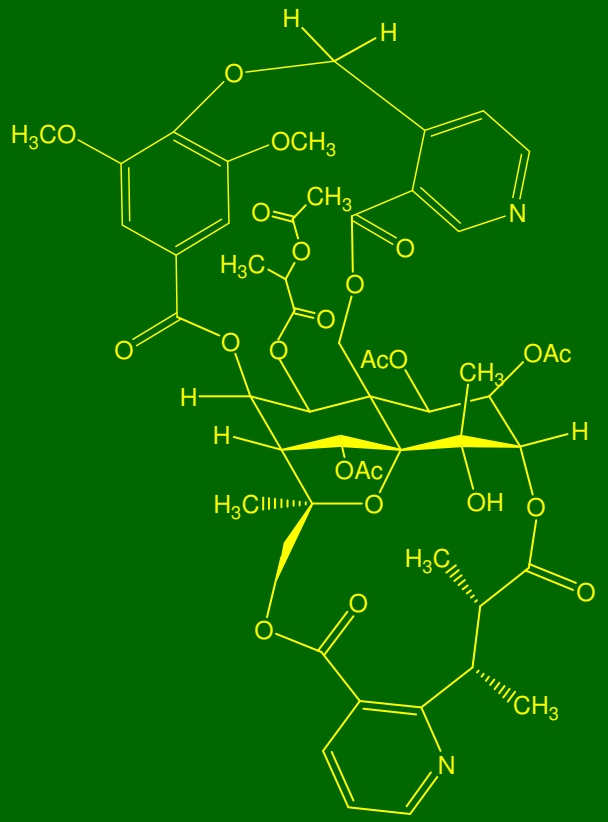


(+)-Amphetamine



'Ecstasy'

Khat constituents - cathedulins



Cathedulin K11

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

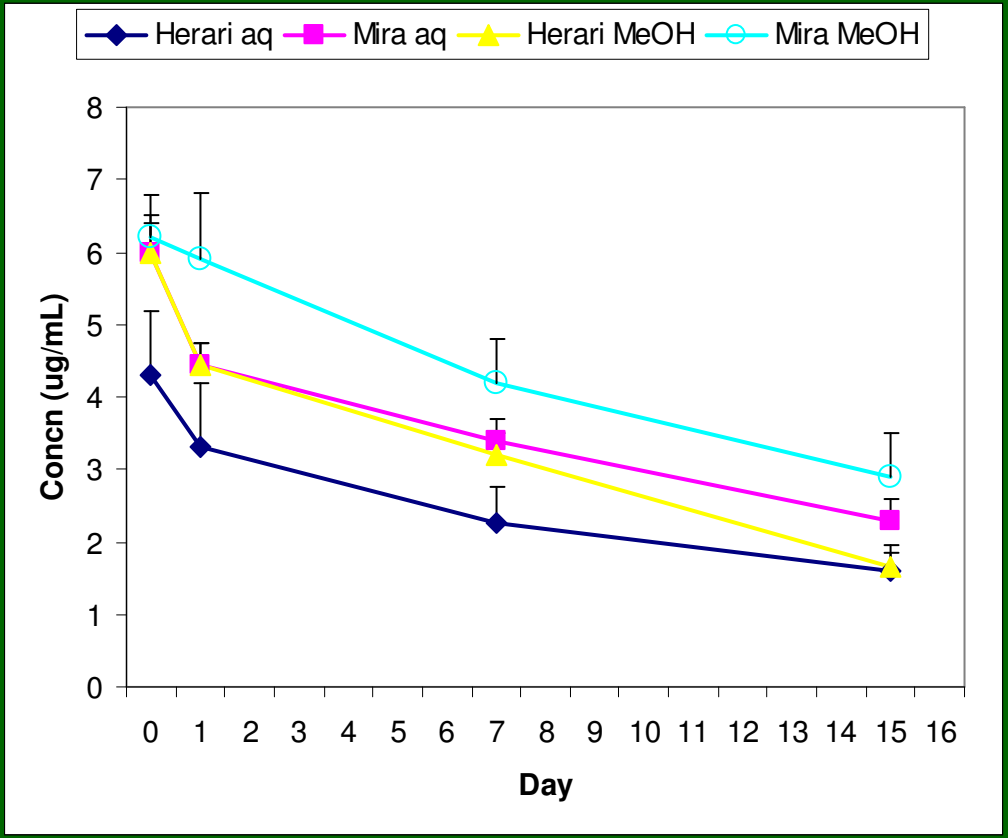
Khat constituents – effect of storage

- 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

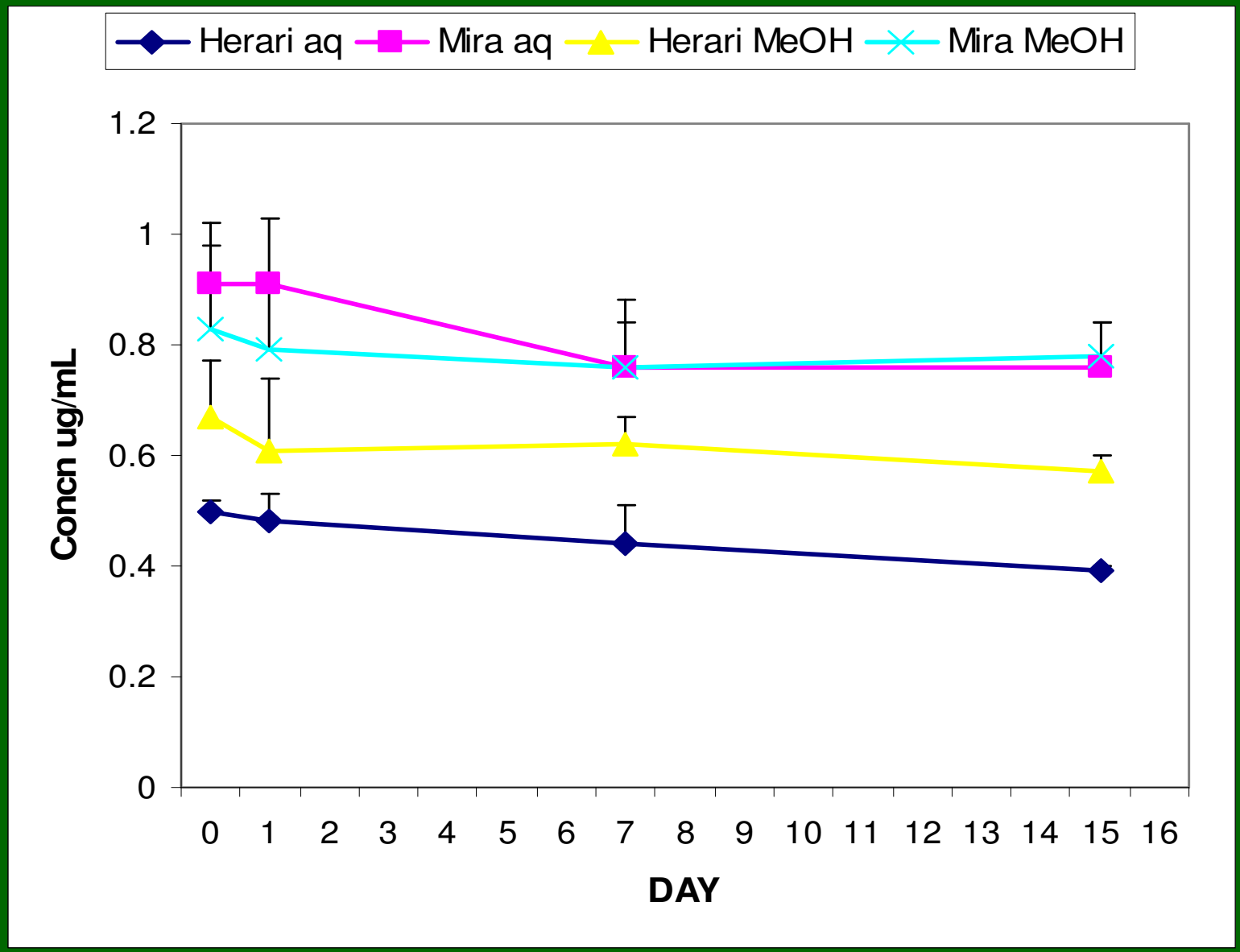
What happens on storage?

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

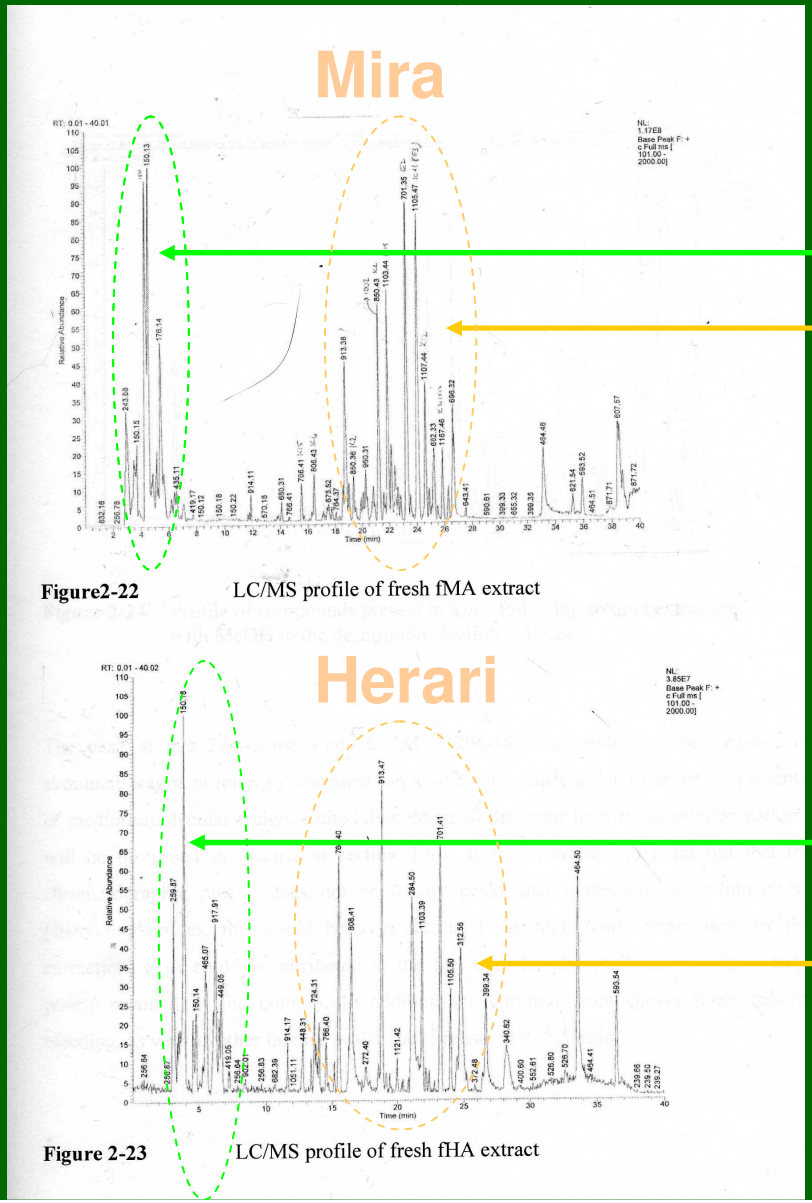


Figure2-22 LC/MS profile of fresh fMA extract

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

Phenylalkylamines

Cathedulins

Phenylalkylamines

Cathedulins

Khat constituents variation

Cathinone, norephedrine and cathedulin K11 quantified (µg/mL)* in extracts using HPLC

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

* Mean ± 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?

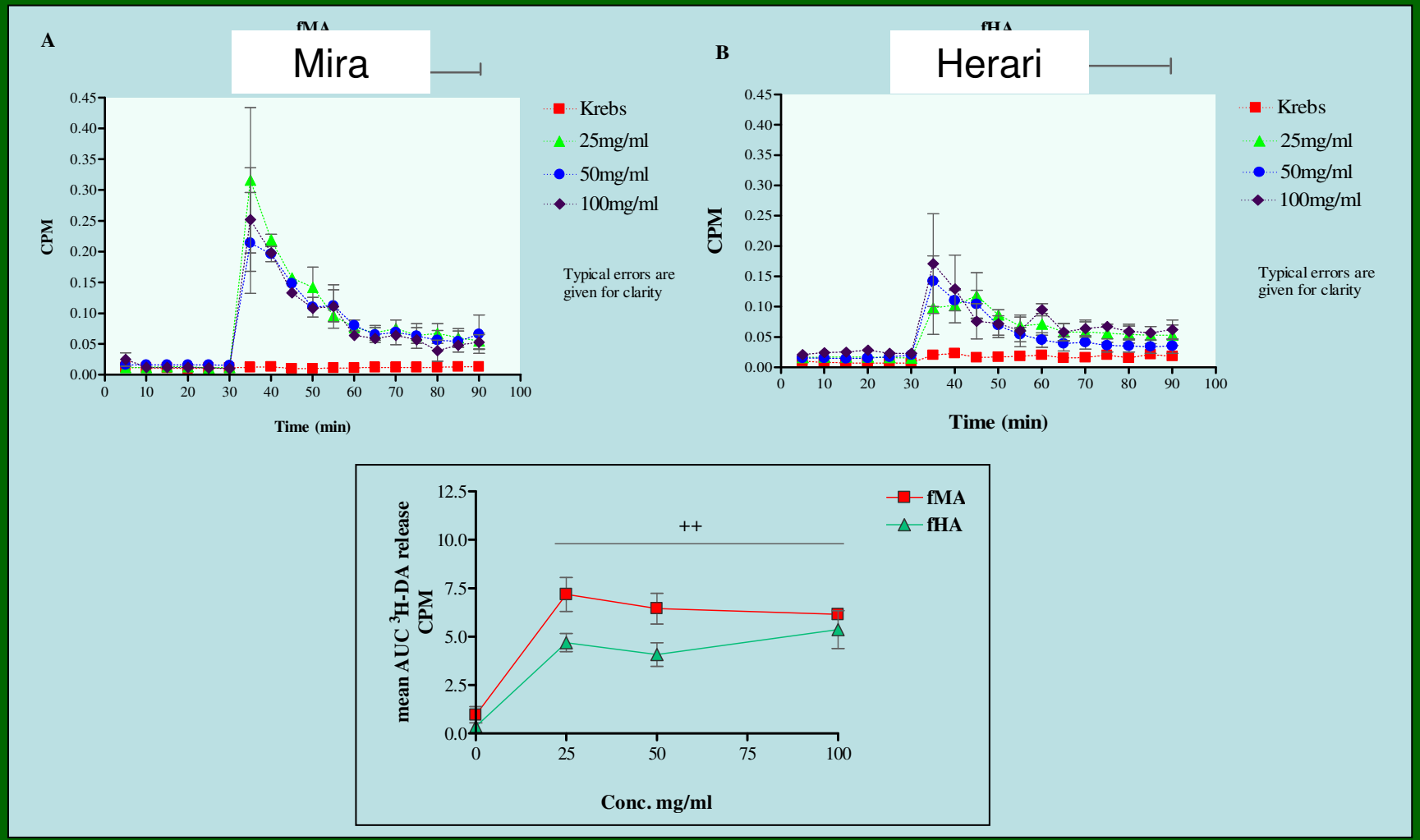
Khat constituents and dopamine

- 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

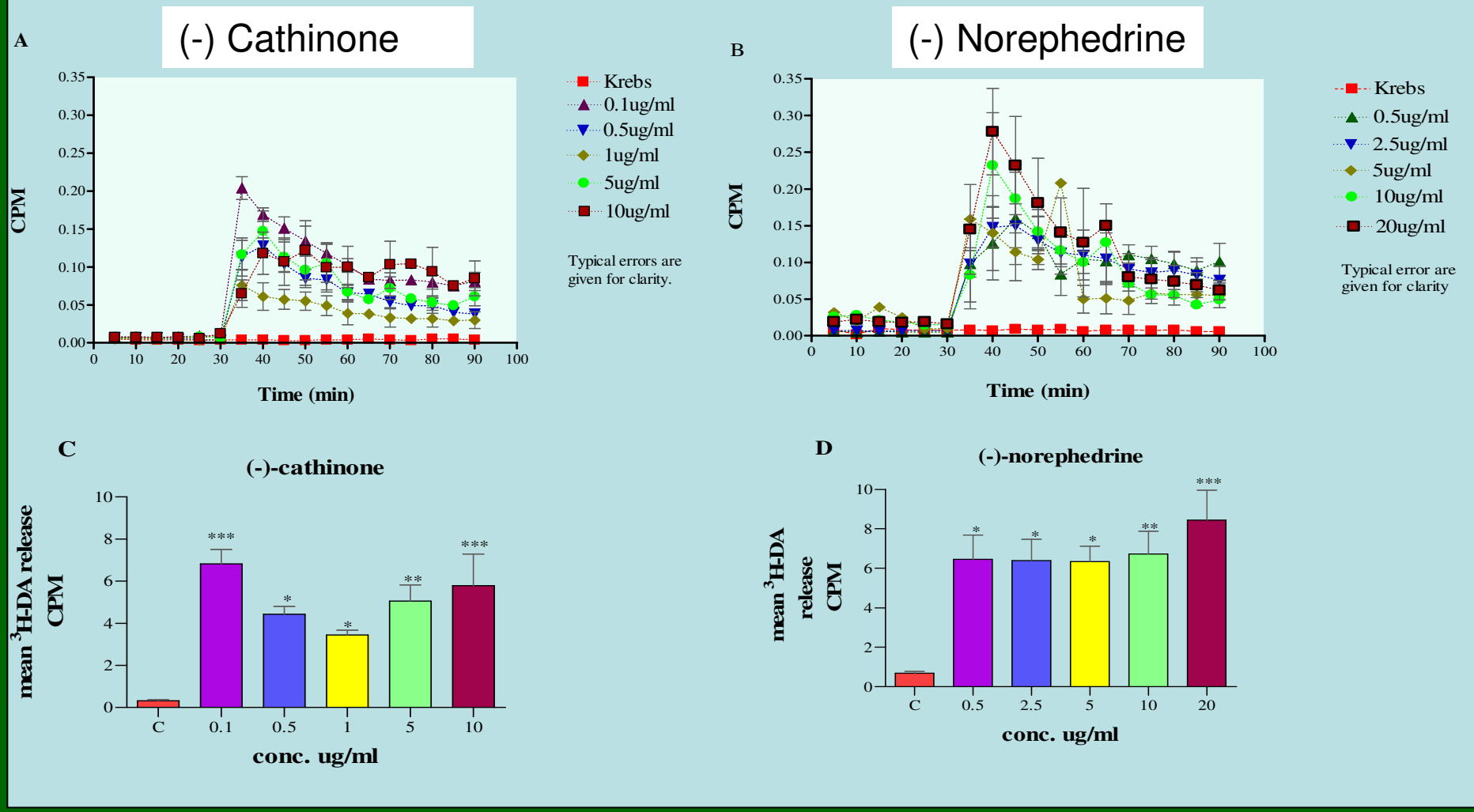
Measuring DA release

- Corpus striatum removed from brains of killed rats
- Slices made - 300μm thick
- Tissue incubated in ^{3}H dopamine (^{3}H DA) – some incorporated into cells
- Washed with Krebs solution- removes excess DA
- Slices put in superperfusion 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

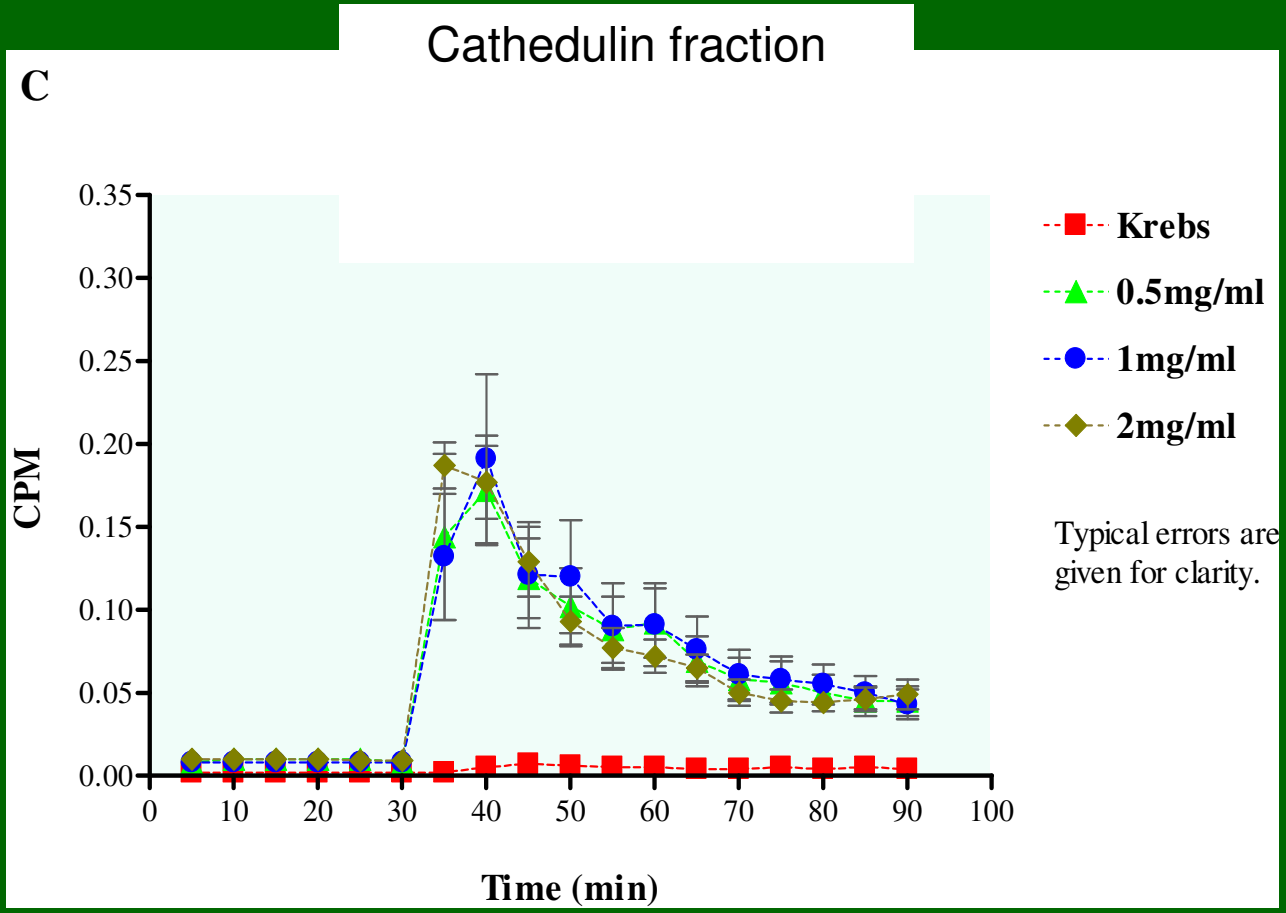
Dopamine (DA) release from striatal slices over 100 mins from aqueous extracts



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

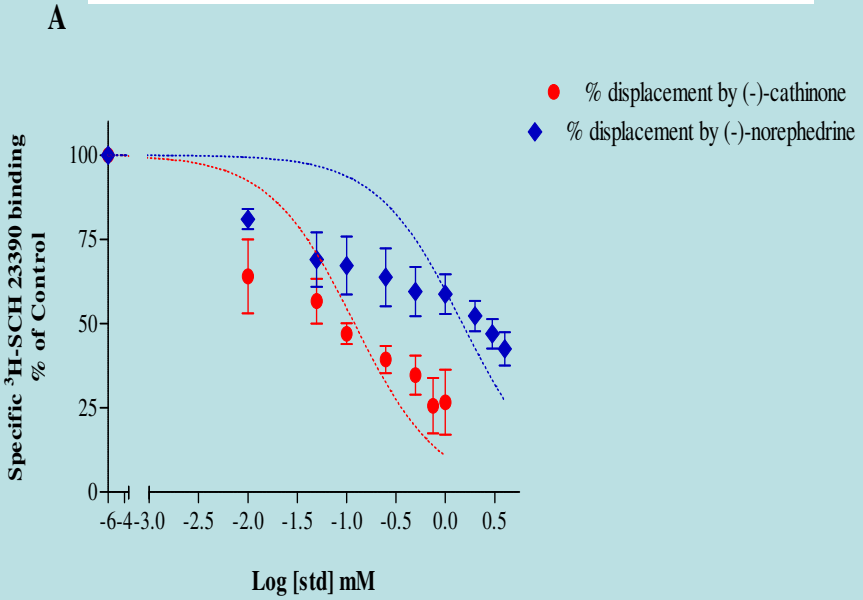
- Uses measurement of displacement of radiolabelled selective binder to receptor
- Two types of DA receptor D1 and D2
- D1 – basis for many psychomotor effects, cognitive function, cardiovascular function. Selective binder is SCH 23390
- D2 – Implicated in several neurological and psychiatric disorders 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

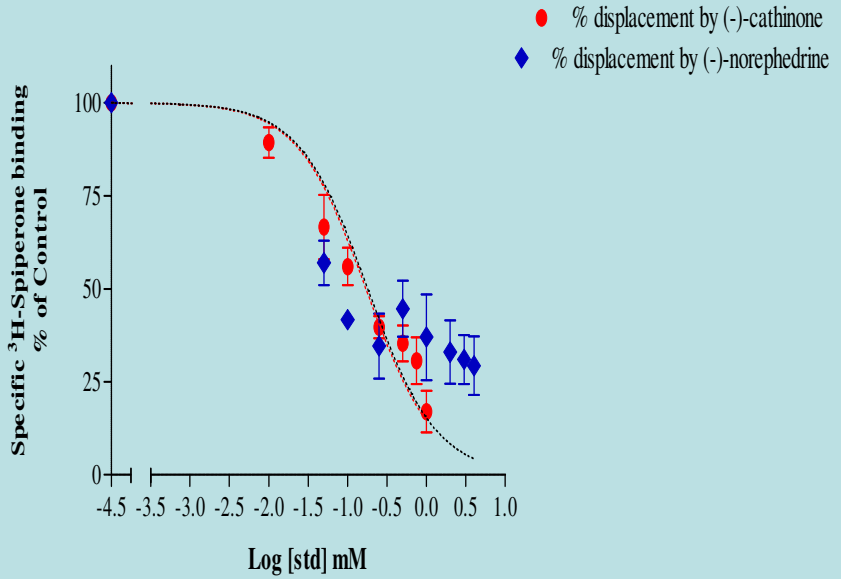
- Corpus striatum removed from brains of killed rats
- Striata homogenised in TRIS buffer
- Washed several times and centrifuged to remove supernatant
- Pellet re-suspended in buffer and range of concentrations of test substance and constant dose of known activity ^{3}H-labelled selective binder added
- Incubated at 37º 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}H-SCH23390 D1 receptor



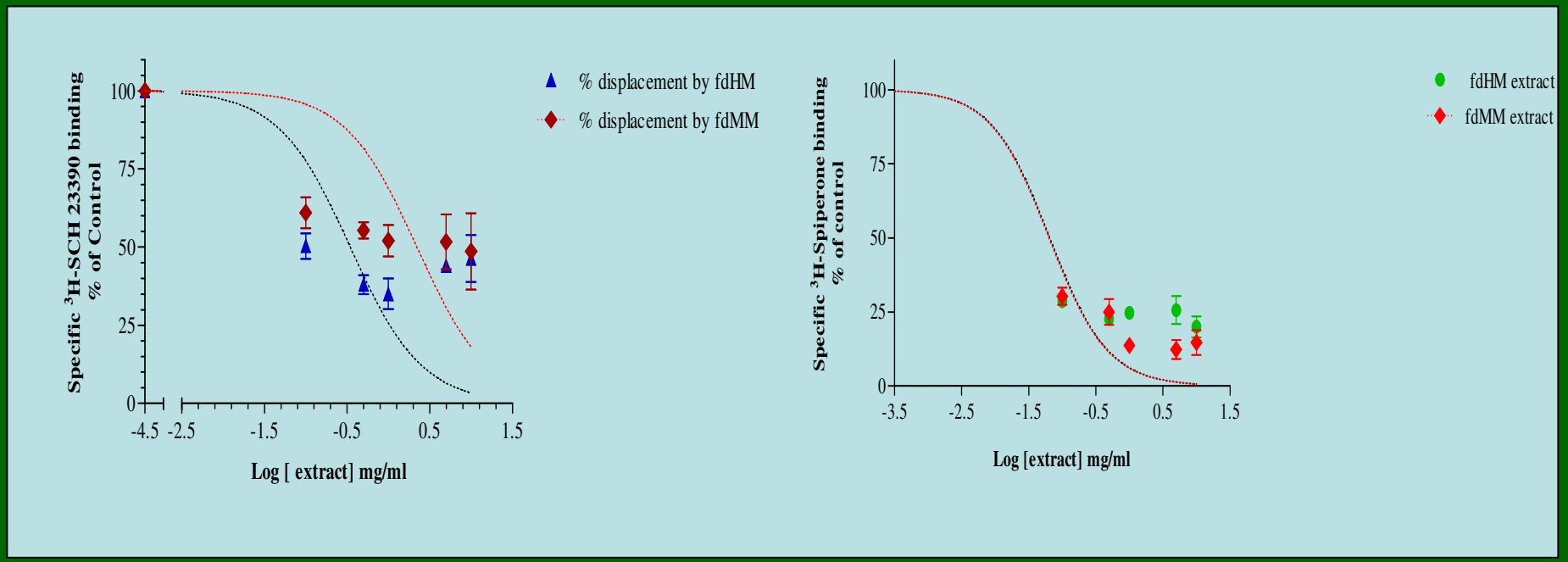
^{3}H-Spiperone D2 receptor



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

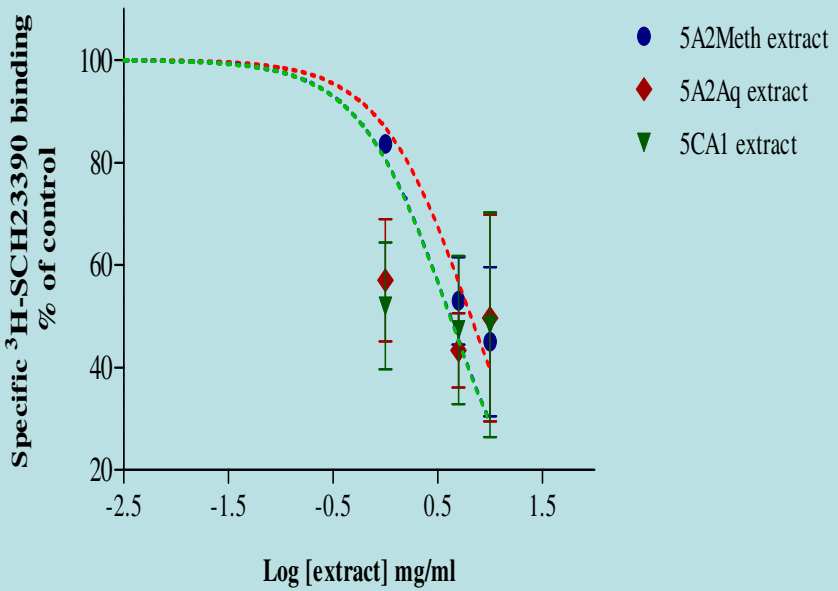
^{3}H-SCH23390 D1 receptor

^{3}H-Spiperone D2 receptor

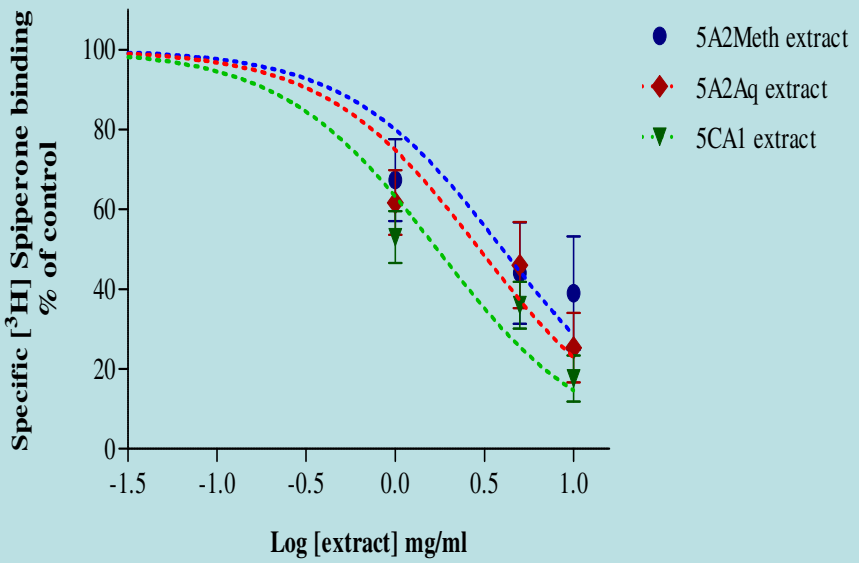


Displacement of selective receptor binding compounds by cathedulin fractions

^{3}H-SCH23390 D1 receptor



^{3}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!