

Author's response to reviews

Title: Distinct single cell signal transduction signatures in leukocyte subsets stimulated with khat extract, amphetamine-like cathinone, cathine or norephedrine

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Author's response to reviews: see over
We hereby submit a revised version of the submitted article entitled: "Distinct signal transduction signatures in leukocyte subsets stimulated with the khat-derived amphetamine-like cathinone and its derivatives cathine and norephedrine" by Therese Bredholt, Elisabeth Ersvær and coworkers, for consideration in your journal.

We are grateful for the reviewers’ comments and have done our best to address the concerns raised by the reviewers. A detailed point-to-point reply to the referees' comments is given below. In addition, a revised manuscript is attached to this document. All substantive changes are highlighted in yellow.

We hope that you will find our manuscript improved by this revisions and we hope that you will now find it suitable for publication in BMC Pharmacology and Toxicology.

Yours sincerely,

Bjørn Tore Gjertsen
Therese Bredholt
Elisabeth Ersvær
Reviewer: Sanir Aleryani

1) The author did not describe in details of khat extracts preparations, it will be useful to identify:i) source of khat (purchased...etc.), ii) the age of khat since it was cultivated or obtained and storage conditions (i.e. temperature) until testing, iii) how much khat was used in grams, type of leaves used (soft versus hard ones, iv) and whether or not the stems of the plant were used in the preparation of the extracts or not.

*Khat (asili cultivate) from the Meru district in Kenya was purchased in Nairobi, transported at 4°C under moist conditions, and the material was extracted within 48 hrs of harvest with methanol [1, 2]. The khat-extract was prepared from approximately 200 grams of khat, using the leaves and the soft parts of the twigs, which were chopped into small pieces (5 mm) and covered with methanol. The methanol extract was dried using a rotary evaporator, the semi-solid residue dissolved at a concentration of 0.2 g/ml in dimethylsulphoxide (DMSO; Sigma, St. Louis, MO, USA) and the stock solution stored at -80°C. This information is now included in the manuscript (see page 14-15).*

In addition, the authors didn’t specify how they obtained khat and cathinone; khat plant is inhibited in Norway while cathinone from sigma is a scheduled 1 drug and its sale and export is restricted.

*In order to import and study khat, which is classified as an illegal narcotic substance in Norway, written permission was obtained from The Norwegian Medicines Agency (2002-07-08, reference 01/705). This information is now included in the manuscript (see page 15). In order to import and study cathinone, its sale and export is restricted in Norway, written permission was obtained from The Norwegian Medicines Agency (see page 14).*

2) While the authors referenced the method by which they extracted khat, it would be of interest to the readers of this paper to show in detail how the quality of the extracts was determined i.e., presence of cathinone and other khat-derived materials, please clarify this.

*The stock solution was diluted in Roswell Park Memorial Institute (RPMI)-1640 medium (Sigma-Aldrich) with 10% fetal bovine serum (FBS), 2 mM L-glutamine and antibiotics (100 IU ml-1 penicillin and 100 µg ml-1 streptomycin; Gibco, Grand Island, NY, USA), and precipitates removed by centrifugation (10 000 g, 15 minutes, 4oC) [2]. The khat-extract supernatant was added experimental cell cultures giving final dilutions of 10-3 and 3.16 x 10-4, based on previous studies [2]. All experimental cell cultures contained 0.1% DMSO.*

*Cathinone is relatively unstable, being transformed to cathine upon wilting of khat leaves, while being metabolized predominantly to norephedrine in vivo [3, 4]. Cathinone, cathine and norephedrine are therefore suited as reference substances, indicating the freshness of the khat sample and the stability of the khat-extract. The concentrations of cathinone, cathine and norephedrine in the khat-extract were determined using high pressure liquid chromatography and mass spectrometry (HPLC-MS-MS), as previously described [2], and the values are displayed in Table 1, together with experimental cell culture concentrations. This information is now better clarified in the manuscript (see page 15).*

Khat can induce apoptosis as the authors presented but there was no mention of the mechanism by which apoptosis was generated. One hypothesis is by increased free radicals as proposed by reference 30 in their reference list and by Aleryani etc. (Khat a drug of abuse: roles of free radicals and antioxidants). It would be useful if the authors can suggest a mechanism in the discussion section for their apoptosis observation based on the above two references.
Different hypothesis regarding khat and the induction of apoptosis is now better clarified in the discussion (see page 10). We have included the reference Aleryani et al.

The author states that “We report that the khat-derived amphetamine cathinone”. This statement is confusing, and my problem is that the combination of khat, amphetamine and cathinone in one sentence “khat-derived amphetamine cathinone” didn’t make sense. I think the authors are trying to state that cathinone is the derived chemical and that it is an amphetamine-like compound, and thus the statement should reflect this fact and be revised to “khat-derived amphetamine-like cathinone) OR (Khat-derived cathinone). Please consider this revision and make appropriate changes in the title as well.

The authors agree regarding the somewhat confusing statement. Throughout the manuscript the statement are revised to either “khat-derived amphetamine-like cathinone” or “khat-derived cathinone”.

In addition, I advise the authors to depict a figure which summarize their findings in this and previous studies conducted by their group; this is relevant to the followers of khat research.

A summarizing figure is now included in the manuscript.

Reviewer: Valentina Galbiati

Minor essential revision

DISCUSSION

You write "Cathinone and its natural derivates generally suppressed basal phosphorylation of the examined signal transducers and stress sensors, while khat-extract induced protein post-translational modification" and after "Further, even if not statistical significant, cathine and cathinone indicated elevated p-CREB levels in all leukocyte subsets, whereas nor ephedrine reduced basal phosphorylation of CREB". Have you got some more explanation for the CREB's phosphorylation?

Our results may indicate that the alkaloids cathine and cathinone can induce CREB phosphorylation as has been shown for e.g. Ephedrine (Eph). Eph, is an alkaloid chemically similar to cathinone, and has been shown to induce PKA-mediated cyclic AMP response element-binding protein (CREB) phosphorylation most probably through β-adrenergic receptor/cyclic AMP/PKA/DARPP-32 signaling pathway [5]. This information is now included in the manuscript (see page 12), however, we stress that our results are statistically non-significant.

As you certainly know, the herbal drug kath contains other compounds like Meruchatine, a phenylalkylamine (brenneisen et al 1984), and also catheduline alkaloids (Crombie, 1980). Have you considered these others compounds and their pharmacological/biological actions? And there are other natural plants or phytocomplexes with the same characteristics of Catha edulis?
The authors are aware of that khat contains several other bioactive compounds. However, the main aim of our study was to address the khat-derived amphetamine-like alkaloids. It would be very interesting though, in another study, to consider these other natural compounds.

References