EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee on Herbal Medicinal Products (HMPC)

Addendum to Assessment report on *Aloe barbadensis* Mill. and *Aloe* (various species, mainly *Aloe ferox* Mill. and its hybrids), folii succus siccatus

Rapporteur(s)	J. Wiesner
Peer-reviewer	H. Foth / I. Chinou / H. Pinto Ferreira

HMPC decision on review of monograph *Aloe barbadensis* Mill. and *Aloe* (various species, mainly *Aloe ferox* Mill. and its hybrids), folii succus siccatus adopted on 22 November 2016	24 November 2021
Adoption by Committee on Herbal Medicinal Products (HMPC)	26 January 2022

Review of new data on *Aloe barbadensis* Mill. and *Aloe* (various species, mainly *Aloe ferox* Mill. and its hybrids), folii succus siccatus

Unscheduled review

Data submitted by J. Wiesner to HMPC on 26 January 2022

- ☐ Safety data
- ☒ Other scientific data (*in vivo* Comet assays with aloe-emodin and *Aloe ferox* juice)
- ☐ Regulatory practice
- ☐ Referral
- ☐ Other

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Availability of new information (i.e. likely to lead to a relevant change of the monograph)

Scientific data	Yes	No
New non-clinical safety data likely to lead to a relevant change of the monograph	☐	☒
New clinical safety data likely to lead to a relevant change of the monograph	☐	☒
New data introducing a possibility of a new list entry	☐	☒
New clinical data regarding the paediatric population or the use during pregnancy and lactation likely to lead to a relevant change of the monograph	☐	☒
New clinical studies introducing a possibility for new WEU indication/preparation	☐	☒
Other scientific data likely to lead to a relevant change of the monograph	☐	☒
Regulatory practice	Yes	No
New herbal substances/preparations with 30/15 years of TU	☐	☒
New herbal substances/preparations with 10 years of WEU	☐	☒
Other regulatory practices likely to lead to a relevant change of the monograph	☐	☒
Referrals likely to lead to a relevant change of the monograph	☐	☒
New / Updated Ph. Eur. monograph likely to lead to a relevant change of the monograph	☐	☒
Consistency	Yes	No
New or revised public statements or other HMPC decisions likely to lead to a relevant change of the monograph	☐	☒
Relevant inconsistencies with other monographs within the therapeutic area that require a change of the monograph	☐	☒
Other relevant inconsistencies that require a change of the monograph	☐	☒

Summary and conclusions on the review

During the review, three new references not yet available during the first/previous assessment were identified.

Three references were considered to be relevant for the assessment.

No references justify a revision of the monograph.

No revision is considered required because there is no references that would trigger a change in the existing wording of the monograph in section 5.3 Preclinical safety data.

Scientific data

Non-clinical toxicology:

Nesslany *et al*. (2009) performed an *in vivo* mouse comet assay on both isolated kidney and colon cells in order to demonstrate a possible organospecific genotoxicity after oral administration of aloe-emodin (AE). Furthermore, an Ames test and an *in vitro* micronucleus assay with TK6 human lymphoblastoid cells were performed in their microscale version both with S9 from Aroclor 1254-induced liver or kidney, and without S9.

AE induced primary DNA damage in the liver and in the kidney as observed between 3 and 6 hours after two oral administrations at 500, 1000 and 2000 mg/kg bw, underlining an *in vivo* genotoxic mechanism of action. Furthermore, AE induced a clear genotoxic activity both in the Salmonella typhimurium strains TA1537 and TA98 and in the *in vitro* micronucleus assay in the absence as well as

in the presence of metabolic activation. As no significant variation in the genotoxic activity of AE was noted when using either liver or kidney S9-mix, it seems that no quantitatively and/or qualitatively specific renal metabolism occurs. The kidney may be a target organ of AE as it is the major route of excretion. The authors concluded that AE present in plant extracts should be considered as an *in vivo* genotoxin and this property should be taken into account in the risk assessment for human exposure.

Assessor's comment:

In the study Nesslany et al. (2009) the effects on kidney cells were only seen at highest dosage. Therefore, dose relation-ship is questionable and according to OECD 489, the response is neither clearly negative nor clearly positive. Also for the effects on the colon cells, there is no clear dose-response relationship. Therefore, according to the OECD 489, these results should also be interpreted with caution. With this, not all 3 conditions needed for a positive result according to OECD 489 are met. In OECD 489, it is mentioned: "In case the response is neither clearly negative nor clearly positive (i.e. not all the criteria listed in paragraphs 59 or 60 are met) and in order to assist in establishing the biological relevance of a result, the data should be evaluated by expert judgement and/or further investigations conducted, if scientifically justified.". From our point of view, the absolute conclusion drawn by the authors could therefore not be followed without any doubt. Therefore, the results could at most be seen as a "trend".

Galli *et al*. (2021a) conducted a new *in vivo* study (*in vivo* alkaline comet assay in mice -OECD 489) to test the potential genotoxicity of aloe-emodin at doses of 250, 500, 1000 and 2000 mg/kg bw/day on preparations of single cells from the kidney and colon of treated male mice. Following treatment with the test item, no clinical signs were observed in animals in any treatment group. Slight bodyweight loss was randomly observed in all groups treated with the test item and was more evident in the groups dosed at 1000 and 2000 mg/kg bw/day. Under these experimental conditions, aloe-emodin showed no genotoxic activity. The authors mentioned that possible oxidative damage to colon tissues could not be excluded based on the results obtained after repair enzyme treatment and they hypothesised that the mechanism of action for HADs is more to be seen in a tumour promoting effect at a diarrheagenic doses, rather than a mechanism mediated by a genotoxic effect.

Assessor's comment:

Aloe-emodin did not induce DNA damage in preparation of single cells from colon and kidneys following oral gavage at doses of 250, 500, 1000, and 2000 mg/kg/day under the standard reported experimental conditions. Furthermore, no statistically significant increases in tail moment and tail intensity were observed over those in the vehicle-treated control group at any dose level. For colon tissue (kidney cells were negative), following the enzymatic treatment, statistically significant increases in break sites were observed above 500 mg/kg bw/ day, although no dose-response relationship was identified.

Galli *et al*. (2021b) investigated by using the *in vivo* alkaline comet assay in animals (OECD 489), the potential *in vivo* genotoxicity of dried *Aloe ferox* juice (commercial sample, containing 0.2% aloe-emodin; no information about Ph. Eur. compliance) at dose levels of 500, 1000, and 2000 mg/kg/day in mice (corresponding to 1, 2, and 4 mg/kg/day aloe-emodin). The juice showed no genotoxic activity in preparations of single cells from the colon of the treated Hsd:ICR (CD-1) male mice. No statistically significant increase in DNA migration over the negative control was observed by analysis of variance for both comet parameters, tail moment and tail intensity, apart from the positive control ethyl methanesulphonate that induced clear and statistically significant increases in DNA migration parameters over the concurrent controls. The authors concluded that dried *Aloe ferox* juice containing hydroxyanthracene derivatives does not induce DNA damage in preparations of single cells from colon

in *in vivo* comet genotoxicity studies and they suggested that the hyperplastic changes and mucosal hyperplasia observed after long-term administration of *Aloe vera* non-decolourised whole leaf extract may be attributed to an epigenetic effect of the material under investigation.

Assessor's comment:

Aloe ferox juice did not induce DNA damage in preparation of single cells from colon following oral gavage at doses of 500, 1000, and 2000 mg/kg/day under the standard reported experimental conditions. Furthermore, no statistically significant increases in tail moment and tail intensity were observed over those in the vehicle-treated control group at any dose level.

General assessment in relation to the monograph of the HMPC

Overall, the results of the three *in vivo* studies do not trigger an unscheduled revision of the monograph since results of the Comet assays on aloe-emodin revealed no, or inconclusive, genotoxic effects.

To ensure that the potential genotoxic suspicion can be clearly eliminated, more experimental data on characterised materials are needed. Until genotoxic effects are ruled out without doubt, also the contraindications etc. should be kept.

References

a) References relevant for the assessment:

Nesslany F, Simar-Meintières S, Ficheux H, Marzin D. Aloe-emodin-induced DNA fragmentation in the mouse *in vivo* comet assay. *Mutat Res.* 2009, 678(1):13-9, in press, doi: 10.1016/j.mrgentox.2009.06.004

Galli CL, Cinelli S, Ciliutti P, Melzi G, Marinovich M. Aloe-emodin, a hydroxyanthracene derivative, is not genotoxic in an *in vivo* comet test. *Regul Toxicol Pharmacol.* 2021(a), 124:104967, in press, doi: 10.1016/j.yrtph.2021.104967

Galli CL, Cinelli S, Ciliutti P, Melzi G, Marinovich M. Lack of *in vivo* genotoxic effect of dried whole *Aloe ferox* juice. *Toxicol Rep.* 2021(b), 8:1471-1474, in press, doi: 10.1016/j.toxrep.2021.07.023

b) References that justify the need for the revision of the monograph:

None

Rapporteur's proposal on revision

☐ Revision needed, i.e. new data/findings of relevance for the content of the monograph

☒ No revision needed, i.e. no new data/findings of relevance for the content of the monograph

HMPC decision on revision

☐ Revision needed, i.e. new data/findings of relevance for the content of the monograph

☒ No revision needed, i.e. no new data/findings of relevance for the content of the monograph

The HMPC agreed not to revise the monograph, assessment report and list of references on *Aloe barbadensis* Mill. and *Aloe* (various species, mainly *Aloe ferox* Mill. and its hybrids), folii succus siccatus, by majority.

The following members did not agree with the decision of the HMPC and were of the position that there are new data/findings of relevance for the content of the monograph and a revision is needed: Wojciech Dymowski, Poland.