Author's response to reviews

Title: Isorhamnetin 3-O-robinobioside from Nitraria retusa leaves enhance antioxidant and antigenotoxic activity in human chronic myelogenous leukemia cell line K562

Authors:

jihed boubaker (bj198024292600@yahoo.fr)
mohamed ben sghaier (medbensghaier@gmail.com)
inges skandrani (ines20101979@yahoo.fr)
kamel ghedira (ghe.kamel@yahoo.fr)
leila chekir-ghedira (che_leila@yahoo.fr)

Version: 7 Date: 4 July 2012

Author's response to reviews: see over
Dear Editor,

I have the pleasure to resubmit for publication in your journal: “BMC Complementary and Alternative Medicine”, the revised version of our paper with its revised title: “Isorhamnetin 3-O-robinobioside from Nitraria retusa leaves enhance antioxidant and antigenotoxic activity in human chronic myelogenous leukemia cell line K562” by: Boubaker Jihed, Ben Sghaier Mohamed, Skandrani Ines, Ghedira Kamel, Chekir-Ghedira Leila. Referenced: ID: 9718042267230860.

Responses to Reviewer 1:

1- Title
As proposed by the reviewer I changed the title to “Isorhamnetin 3-O-robinobioside from Nitraria retusa leaves enhance antioxidant and antigenotoxic activity in human chronic myelogenous leukemia cell line K562”

2- Methods
As proposed by the reviewer I changed the section “Assay for cytotoxic activity” to “The cytotoxicity of N. retusa extract and I3-O-Rob against the K562 cells were estimated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, previously described by Boubaker et al, (10).”

As proposed by the reviewer I deleted the figure 2, 3 and 5.
3- Discussion

The reviewer said “This paper presents a research interest in the evaluation of the antioxidant effect of flavonoids and antigenotoxic inside cells, for this reason the discussion part in relation with the cytotoxicity of flavonoids can be summarized to reduce the discussion.” I agree with the reviewer and I changed the section “Some studies have shown that flavonoids (21), polyphenols (22) and tannins (23) are able to altering proliferation of cancer cell. Previous studies have shown that flavonoids induce apoptosis of various tumor cells including K562 cells. This effect has also been observed in other tumor cell lines from gastric, colon and lung carcinomas (24). In addition, flavonoids also inhibited tumor growth through cell cycle arrest and induced apoptosis through a p53-dependent mechanism (25). The presence of the B ring in I3-O-Rob structure should be one of the cytotoxicity factors of I3-O-Rob. In fact, according to Plochmann et al. (26) B ring flavonoids, were more cytotoxic than their structural analogues without the B-ring. This cytotoxicity should be also related to the presence of the OH group, precisely at C4’ of B-ring of the tested flavonoid. This hypothesis is supported by the studies of Michels et al. (27) and Agullo et al. (28) who showed that C’4 hydroxylated flavonoids are more cytotoxic than methylated ones. Likewise, we believe that the presence of methoxy group on C3’ of isorhamnetin moiety participates to the cytotoxicity of I3-O-Rob. In fact methoxylation was described to increase the hydrophobicity of flavonoids and consequently their cytotoxicity (29). We have shown here that O-methylation as well as glucuronidation result in an increased cytotoxicity (26). Kawai et al. (29) studied structure–activity relationships of four flavonoids by comparing their antiproliferative effects. They concluded that the C2–C3 double bond and 3-hydroxyl group of ring A are important factors for antiproliferative activity. Brusselmans et al. (30), reported that the presence of carbonyl function on C4 of ring C may be involved in the cytotoxicity of such molecules. It also has been advanced that flavonoids with such carbonyl function induce inhibition of fatty acid synthesis and thus, population growth arrest and cell death. We believe so for I3-O-Rob cytotoxic. Likewise, we believe that the presence of sugar moiety in glycosylated flavonol, may participate in reducing the proliferation of several types of cancer cells. Indeed, Akland et al. (31) reported that the flavonol glycosides tend to decrease the expression of specific nuclear antigens along with reducing intercellular protein levels in both breast and intestinal cancer cells. The flavonoids may intervene at different levels of lipid peroxidation process (32).
are able to capture directly the radical species and thus interrupt the step of propagating radical (33). Also, being good chelating, they are capable of coordinating iron free. Finally, flavonoids present on the surface of the membranes are able to regenerate the vitamin E, one of the essential antioxidants in the cell membranes protection (34).” To “Some studies have shown that flavonoids induce apoptosis of various tumour cells including K562 cells. This effect has also been observed in other tumor cell lines from gastric, colon and lung carcinomas (19). In addition, flavonoids also inhibited tumor growth through cell cycle arrest and induced apoptosis through a p53-dependent mechanism (20).

Flavonoids may intervene at the different levels of lipid peroxidation process (21). They are able to directly capture the radical species and thus interrupt the step of propagating radical (22). Moreover, being good chelating, they are capable of coordinating the free iron. Finally, flavonoids present on the surface of the membranes are able to regenerate vitamin E, one of the essential antioxidants in the cell membranes protection (23).”

We tried to address the grammatical and typographical errors.

Responses to Reviewer 2:

1- Methods

Section preparation of ethyl acetate extract from Nitraria retusa leaves

The reviewer said “The sentence “Ethyl acetate extract was screened for the presence of flavonoids, polyphenols and tannins using the methods previously described by Boubaker et al. (14).” Can be deleted because the discussion is not based on chemical composition but on the relationship structure activity.” I agree with the reviewer and I deleted the sentence “The sentence “Ethyl acetate extract was screened for the presence of flavonoids, polyphenols and tannins using the methods previously described by Boubaker et al. (14).”

2- Discussion

The reviewer said “The manuscript evaluates the effect of flavonoids in K562 cells, for this reason, the sentence “Some studies have shown that flavonoids (21), polyphenols (22) and tannins (23) are able to altering proliferation of cancer cell. Previous studies have shown that flavonoids induce apoptosis of various tumor cells including K562 cells. This effect has also been observed in other tumor cell lines from gastric, colon and lung carcinomas (24). In
addition, flavonoids also inhibited tumor growth through cell cycle arrest and induced apoptosis through a p53-dependent mechanism (25).” Can be modified to “Some studies have shown that flavonoids induce apoptosis of various tumor cells including K562 cells. This effect has also been observed in other tumor cell lines from gastric, colon and lung carcinomas (24). In addition, flavonoids also inhibited tumor growth through cell cycle arrest and induced apoptosis through a p53-dependent mechanism (25).” I agree with the reviewer and I changed “Some studies have shown that flavonoids (21), polyphenols (22) and tannins (23) are able to altering proliferation of cancer cell. Previous studies have shown that flavonoids induce apoptosis of various tumor cells including K562 cells. This effect has also been observed in other tumor cell lines from gastric, colon and lung carcinomas (24). In addition, flavonoids also inhibited tumor growth through cell cycle arrest and induced apoptosis through a p53-dependent mechanism (25).” To “Some studies have shown that flavonoids induce apoptosis of various tumour cells including K562 cells. This effect has also been observed in other tumor cell lines from gastric, colon and lung carcinomas (19). In addition, flavonoids also inhibited tumor growth through cell cycle arrest and induced apoptosis through a p53-dependent mechanism (20).”

3- Conclusion
As proposed by the reviewer I deleted the sentence “Knowing that humans ingest about 1 g of flavonoids in their daily diet, and are increasingly associated with cytoprotective antitumour properties, it may encourage, after other confirmatory tests, using the bioactive components of Nitraria retusa as dietary supplements.”

We tried to address the grammatical and typographical errors