Reviewer's report

Title: Activity of Corilagin to treat animal model of acute cholestasis by anti-inflammatory and anti-oxidative pathway

Version: 1 Date: 21 December 2012

Reviewer: Thomas Schreiter

Reviewer's report:

The manuscript entitled “Activity of Corilagin to treat animal model of acute cholestasis by anti-inflammatory and anti-oxidative pathway” submitted by Jin et al. describes positive effects of Corilagin on handling of oxidative stress and the influence of the anti-inflammatory pathway in a rat model of acute cholestasis. The markers assayed were myeloperoxidase and malondialdehyde for oxidative stress and superoxide dismutase responsible for removal of reactive oxygen species (ROS). Nitric oxide (NO) and nuclear translocation of NF-kappaB served as marker for analysis of the anti-inflammatory effect. The results were compared to Ursodeoxycholic acid and Dexamethasone, medications currently used for treatment of cholestatic hepatopathies.

The experiments were carried out carefully, but there are some issues that should be addressed by the authors:

Major Compulsory Revisions

1) The purity of the prepared Corilagin with 62% seems quite low. Can the authors exclude a cooperative effect of any substance in the remaining impurities of 38%?

2) The entire manuscript is full of mistakes regarding use of tenses, prepositions and word choice. At some points, the standards for scientific writing are missing. The authors should look for the help of a native English speaker to improve the quality of their text. This reviewer will only suggest corrections for a few errors (from Material and Methods, p. 7-10):

a. Chemicals and reagents

All chemicals were purchased from … (like PBS and other basic stuff) unless indicated otherwise. Affinity-purified rabbit anti-rat NF-#B p65 was received from Santa Cruz Biotechnology (Santa Cruz, CA, USA)…

Biotin-conjugated goat anti rabbit IgG and streptavidin-horseradish peroxidase (HRP) conjugate are missing. Assay kits were moved to another paragraph, source of ANIT (write out here and not until discussion) and UDCA can also be moved to paragraph “Model and control establishment”.

b. Immunohistochemistry assay
SP (probably Streptavidin peroxidase, write out when used first time) immunohistochemical assay was employed to detect expression of the nuclear translocation of NF-κB. The slides of hepatic tissue were soaked in 3% H2O2-methanol solution for 20 min in order to block endogenous peroxidase. In the next step 1% Triton X-100 was added at 37°C for 5 min, followed by washing with PBS. After incubation with normal goat serum at room temperature for 20 min, rabbit anti-rat NF-κB p65 IgG antibody (dilution; if used undiluted: volume) was added dropwise and the slides were stored at 4°C overnight. The next day slides were washed with PBS and incubated with biotin-conjugated goat anti-rabbit IgG for 30 min at 37°C. After another washing with PBS streptavidin-HRP was added and incubated for 30 min at 37°C. The slides were thoroughly washed with PBS 3 times for 5 min and stained with 3,3′-diaminobenzidine. Following normal dehydration, lucidification and mounting the slides were analysed under microscope model (manufacturer, country) as specified in our previous studies [18, 19, 20, 21] and digital images were captured with camera model (manufacturer, country).

c. Analysis of NO and markers for oxidative stress
Myeloperoxidase (MPO), malondialdehyde (MDA), superoxide dismutase (SOD), and nitric oxide (NO) were quantified by the respective assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the instructions of the manufacturer. The procedures were also described in our previous studies [22, 23].

d. Purity determination of Corilagin
Purity of Corilagin was determined by HPLC. The procedure abided by our previous study [24, 25]. Briefly, a Hanbon-Kromasil 5µm C18 column was used at 30°C with the wavelength of 268nm for detection. The mobile phase was composed of 0.5% phosphoric acid and methyl cyanide with a ratio of 76:24 and the injection volume was 10µl. Corilagin standard substance (purity>99%) was offered by China National Institute for the Control of Pharmaceutical and Biological Products. The purity of Corilagin was calculated to 62.14%.

(Description of the method belongs here and not in Results section.)

3) Figure legends 1-4: Omit liver protective effect at the beginning, this is yet an interpretation of the data and belongs to the discussion. Write just what is shown in the figure.

4) A more precise title would be:
“Anti-inflammatory and anti-oxidative effects of Corilagin in a rat model of acute cholestasis”

Minor Essential Revisions

1) Figure legend 3: Numbers 4A etc. are wrong.
2) Page 6: TBHP should be written out.
This reviewer suggests to decline the manuscript in the current version and encourages the authors to resubmit when all major revisions have been implemented.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.