Reviewer's report

Title: Effect of Uncaria tomentosa extract on purinergic enzyme activities in lymphocytes of rats submitted to experimental adjuvant arthritis model.

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Reviewer: Toshio Kukita

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Authors described the suppressive effect of the extract from Uncaria tomentosa (a giant vine grown in the Amazon rainforest) on the inflammation and on the purinergic enzyme activities in rats with adjuvant-induced arthritis. Development of the novel medicine from natural compounds involved in the traditional medicine is recognized to be important to develop a safe medicine for human body and authors’ trial is interesting. However, author demonstrated only limited data and it seems to be insufficient to address their conclusion from their limited information described in this manuscript. Authors should consider following points to improve this manuscript.

1. Figure 1: Suppression of inflammation parameters by treatment with the Uncaria tomentosa extract (UT-extract) is interesting. What chemical component identified in Figure 2 is the most effective one in this suppression. Although authors described in Discussion that mitraphylline is likely to be the most responsible chemical compound to suppress inflammation, authors should show an experimental evidence to prove it.

2. Figure 3: Authors demonstrated that nucleotide hydrolysis is up-regulated in arthritic rats but it is efficiently suppressed by the addition of UT-extract. Why UT-extract had no effect on nucleoside hydrolysis in control rats? Are there any molecular difference in the enzyme mediating nucleotide hydrolysis between the enzyme from arthritic rats and that from control (normal) rats?

3. Author prepared lymphocyte-rich fraction from blood to evaluate the enzyme activity of nucleotide hydrolysis as well as adenosine deaminase. In this lymphocyte-rich fraction, cell population is quite heterogeneous in which monocytes are also involved in addition to lymphocytes. Authors should compare the difference in the ratio of monocytes, B cells, T cells (helper T cells CD4+, CD8+; regulatory T cells) in arthritic rats and in control (normal) rats. There will be high possibility that lymphocyte-rich fraction from arthritic rats contain high number of T lymphocytes expressing enzyme of nucleoside hydrolysis.

4. In the enzyme assay (Figure 3 and Figure 4), what chemical component involved in the UT-extract suppresses nucleotide hydrolysis? As this is just the in vitro enzyme assay, it would be easy for authors to address.

5. Table 3 and Figure 3: If UT-extract inhibits enzyme for nucleotide
hydrolysis in the lymphocyte-rich fraction, total nucleotide level in serum should be increased. However, authors’ data in Table 1 showed suppression of ATP level in serum of UT-extract-treated arthritic rats. Authors should also explain the suppression of adenosine level in UT-treated arthritic rats (Table 1) as it seems to be a quite important point to understand the decrease in the plasma adenosine level in respect to the suppression of nucleotide hydrolysis by UT-extract.