Online Resource

3Biotech

In vivo COX-2 modulation and metabolite profiling of *Pandanus tectorius* leaves extracts

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The following photographs were the 6th hour post-induction (post-treatment) of carrageenan representing the carrageenan control and PTA 500 mg/kg + carrageenan late sub-groups.

Briefly, the plant extracts were administered through oral gavage to the respective animals 3 days prior to carrageenan induction (Katanić et al. 2016). On the 4th day, 1 hour after oral administration of the test samples to the treatment groups, 0.1 mL of 1.0% (w/v) carrageenan was subcutaneously injected into the sub-plantar side of the left hind paw of the rats. Before (at time 0) and at hourly intervals for 6 hour after carrageenan induction, the paw thickness was measured using digital micrometer. Prior to the actual paw thickness measurement, the rats in each of the major experimental groups were further divided into two sub-groups as initial and late sub-groups. Initial sub-groups consisted of rats that were hourly measured for paw thickness for 2 hours after carrageenan induction, and paws were dissected for future tests after the 2nd hour of measurement. Paw thickness of rats designated at the late sub-groups was hourly measured from the baseline up to the 6th hour post-carrageenan administration, and paws were dissected for future analyses after the 6th hour of measurement.

The sub-groupings (initial and late) performed on the carrageenan-induced rat paw edema were patterned based on the pro-inflammatory effect of carrageenan. The initial phase inflammation (mediated by serotonin, bradykinin, and histamine) occurs at the first 2 hours of carrageenan administration, while the late phase inflammation, mediated by COX-2 and prostaglandins, occurs from the 3rdup to the 6thhour (Necas et al. 2013). Hence, reduction of paw thickness by the plant extracts after 6th hour of carrageenan administration signifies potential anti-inflammatory activity by targeting COX-2 (Figure 1B in the manuscript).While paw thickness measurement serves as a preliminary test only in determining the activity of the plant extracts*in vivo*, cellular level analyses (histopathology and immunohistochemistry) were also done (Figure 2) and documented (Figure 3) to further justify the claim of the study.

Measurement of the rat pawthickness from the 1st up to 6th hour was conducted by one of the authors (CRDM) under the supervision of a licensed veterinarian.

References:

Katanić J, Boroja T, Mihailović V et al (2016) *In vitro* and *in vivo* assessment of meadowsweet (*Filipendula ulmaria*) as anti-inflammatory agent. J Ethnopharm193:627–636

Necas J, Bartosikova L (2013) Carrageenan: A review. Vet Med (Praha)58:187–205

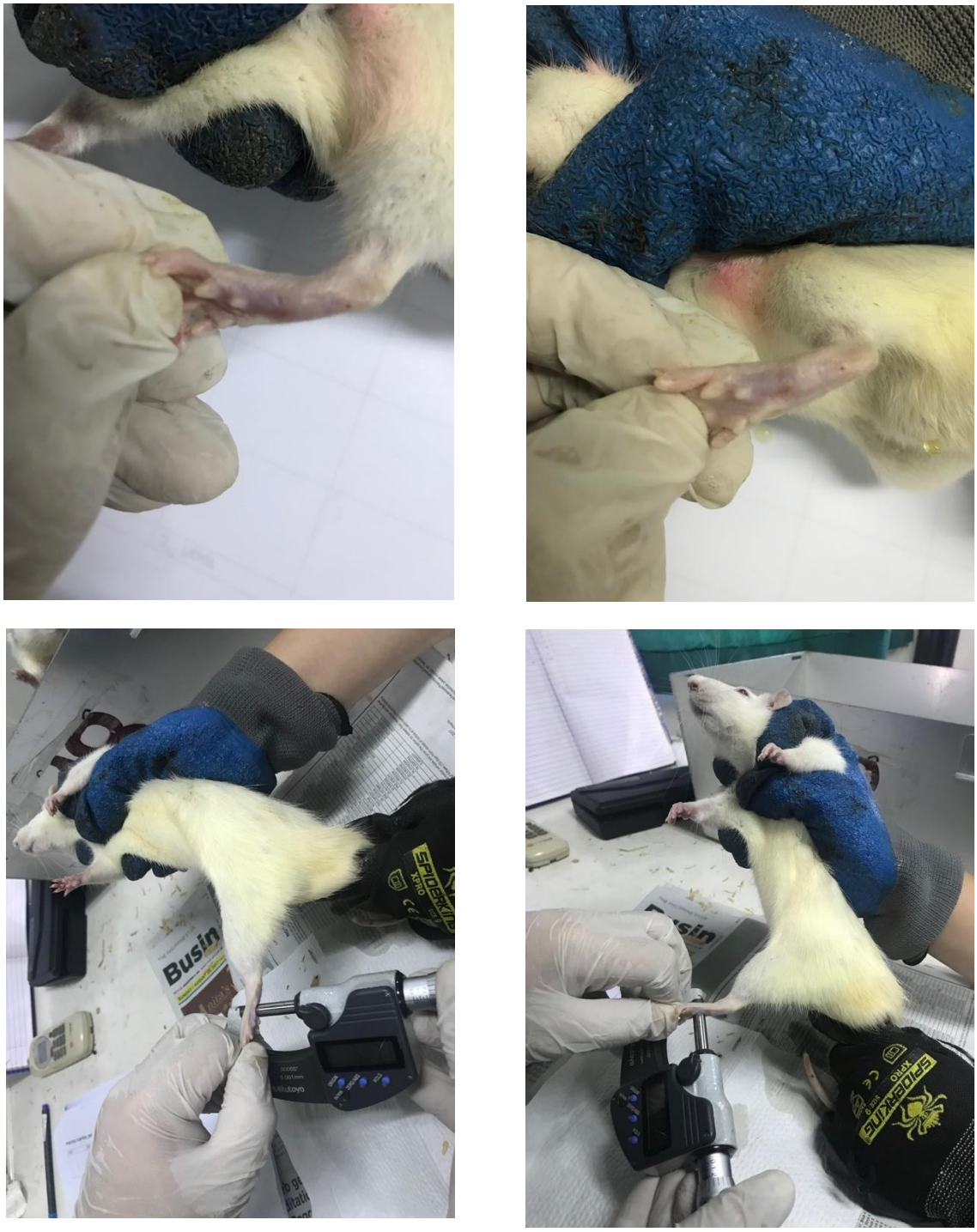

Fig S1 – S4. Sample paw thickness measurement at 6th hour post-induction of carrageenan in rats at late phase sub-groups using digital micrometer. The accuracy of measurement was maximized by having a uniform measuring equipment, use of reference measurement point, and by having the same person who did the measurements.

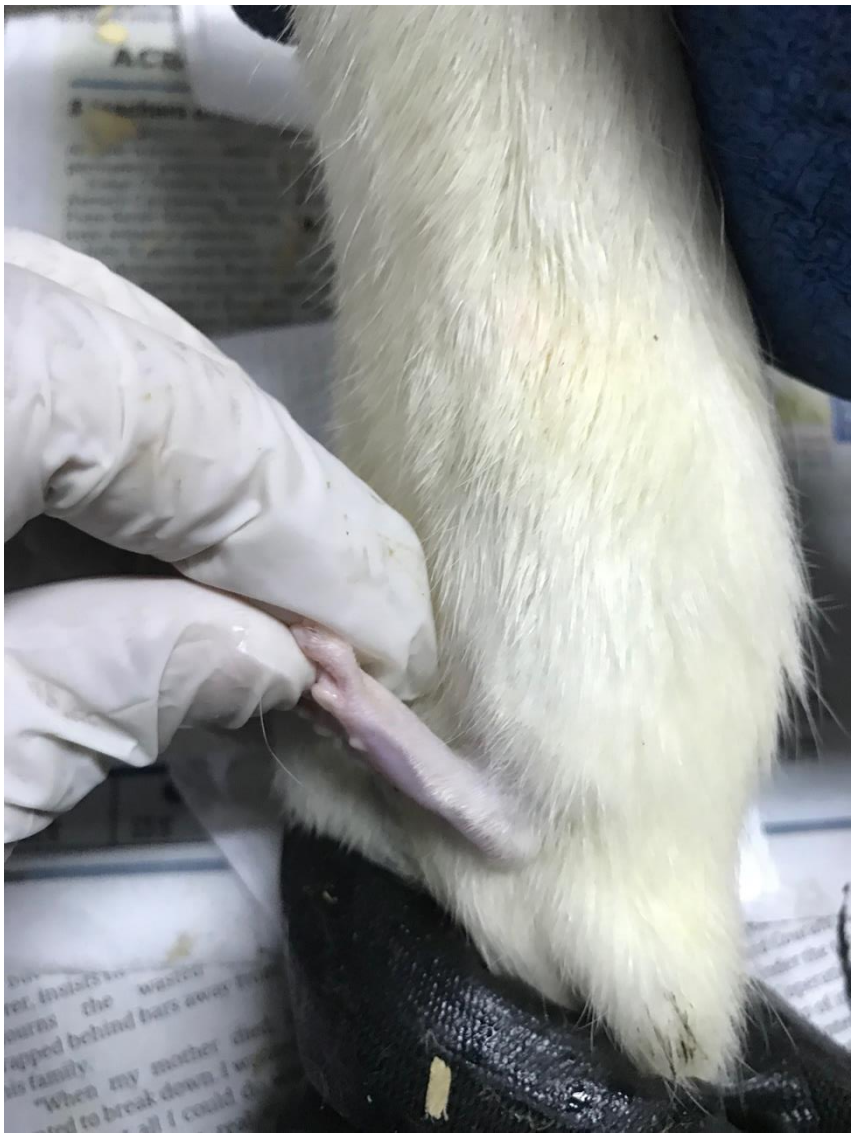

Fig. S5. Paw thickness in the left hind paw of the representative rat from the carrageenan control late sub-group at the 6th hour post-induction of carrageenan. Significant increase in the measured thickness was observed at late-phase inflammation based on the data found in Figure 1B of the manuscript.

Fig. S6. Paw thickness in the left hind paw of the representative rat from PTA 500 mg/kg + Carrageenan late sub-group at the 6th hour post-induction of carrageenan. Significant reduction in paw thickness was observed at the 6th hour post-induction of carrageenan based on the data found in Figure 1B of the manuscript.