



## Research Paper

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# Efficacy evaluation of the formulation of hyaluronic acid–tamarind seed polysaccharide in arthritis rat model

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## ABSTRACT

The purpose of this study was to investigate the rheology and efficacy of the formulation with hyaluronic acid (HA) and tamarind seed polysaccharide (TSP) as a new raw material of the injection for arthritis. First and foremost, the rheological properties of the formulations were investigated with HA and TSP as an analgesic for arthritis and test the improving effect of right/left paw weight ratio in monosodium iodoacetate induced arthritis model. HA formulations with 3 and 4% TSP showed the improved effects on rheological character and reduced the pain on arthritis model. From the rheological data, TSP exhibited the protective effect on the HA formulation against heat sterilization. Tamarind seed polysaccharide is a potential material that can serve as a substitute for hyaluronic acid or a combination with hyaluronic acid in injection for arthritis.

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**Key words:** Hyaluronic acid, Tamarind seed polysaccharide, Storage modulus, Loss modulus, Arthritis.

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## INTRODUCTION

Tamarind seed polysaccharide is obtained from the endosperm of the seed of the tamarind tree, *Tamarindus indica* L., an economically important tree in the Indian subcontinent (Rao et al., 1999). Tamarind seed polysaccharide is a xyloglucan, formed by a linear  $\beta$ -(1,4)-D-glucan backbone, partially substituted at the O-6 position of its glucopyranosyl residues with  $\alpha$ -D-xylopyranose. One of the xylose residues is  $\beta$ -D-galactosylated (Gidley et al., 1991). Xyloglucan water dispersions show high viscosity and broad pH tolerance (Phillips and Williams, 2000). Therefore, they are used as a texturizing agent and a thickener in food and pharmaceutical products (Sahoo et al., 2011). Toxicological studies indicate that xyloglucan is very well tolerated by conjunctival cells and can reduce drug-related toxicity, probably due to its mucin-like structure. Moreover, xyloglucan seems to promote corneal wound healing due to its greater interaction with the integrin recognition system than hyaluronate (Burgalassi et al., 2000). The similarities between xyloglucan and hyaluronic acid, from both physical and biological perspectives, suggest this polysaccharide as a “green” alternative to hyaluronic acid (Khouvilaj et al., 2012).

The hyaluronic acid (HA) in the case of pharmaceutical products, intra-articular injection of HA is a recognized treatment for pain associated with symptomatic knee osteoarthritis (Zhang et al., 2008, 2010). Pharmaceutical products of HA are divided into two major types: native HA products and cross-linked HA products. Native HA products are injected three to five times per one treatment course and its safety has been established based on long time clinical experiences (Peyron, 1993). Native HA has generally been weak to heat or oxidative stress. Recently, much research and development of these products have been carried out to extend the duration in a body, due to the short duration of native HA. To extend the duration of HA by the reaction of cross-link between alcohol group of the HA and an epoxide group of cross-linkers such as 1,4-Butanediol diglycidyl ether, Divinyl Sulfone and 1-ethyl-3-[3-(dimethylamino) propyl] carbodiimide etc.. But these cross-linking agents are known for their toxic chemical (Ishikawa, 2000; Jolanki et al., 1987). In this study, we investigated the rheological properties and analgesic effect against arthritis by formulation with HA and TSP, prepared with a new raw material (TSP) of the injection for arthritis.

## MATERIALS AND METHODS

Xilogel® (MW, 600–700 kDa), Tamarind seed polysaccharide (TSP), is purchased from Indena S.p.A. (Milan, Italy) and Sodium hyaluronate (intrinsic viscosity 2.8 ~ 3.8 m<sup>3</sup>/kg) from Shiseido (Japan) as raw materials for the formulation of arthritis. Monosodium iodoacetate for the arthritis model was from Sigma-Aldrich (Louis, USA). Hyaluronate joint injection (20 mg/2 ml) (Kukje Pharm., Korea) as a positive control of arthritis test was purchased from Wongang Pharm (Korea).

### Animal

Male Sprague Dawley rats (weight 110 – 150 g) were purchased from Japan SLC, Inc. (Hamamatsu, Shizuoka, Japan). The animal housing conditions maintained were 25 ± 1°C, 65 ± 10% relative humidity and 12 h light and dark cycle under conventional conditions in accordance with the institute's animal care guidelines. Food (5L79, Orient-bio Co., Ltd., Korea) and water were available ad libitum. All experiments are designed with 5 heads per each experimental group. The experimental design and research plan along with handling animals and disposal procedure were reviewed by the animal ethics committee and approved by the committee (KJAEK-20180207).

### Rheological analysis

Rheological analyses were performed under continuous and oscillatory flow conditions using a rotational rheometer (Kinexus DSR, Marvern, USA) at fixed temperature 23 ± 0.05°C, equipped with a cone-plate geometry CP50-1 (fixed gap 1 mm). The viscosity at rest ( $\eta_0$ ) was calculated by fitting the flow curves of the different samples, obtained under stationary conditions as a function of shear rate, with the Carreau-Yasuda model that describes the shear thinning behavior of materials. The viscoelastic properties,  $G'$  (elastic modulus) and  $G''$  (viscous modulus), were measured at fixed oscillation amplitude, within the linear viscoelastic region of each material, by varying the oscillation frequency. The trends of  $G'$  and  $G''$  as a function of frequency, measured under linear conditions, are the "mechanical spectra" of the material and describe its structural properties (Lapasin and Pricl, 1995; Yasuda et al., 1981).

### Injectable formulation with HA and TSP

All samples, which were mixed with the crosslinked HA gel and TSP in various ratios, were dispersed in phosphate buffered saline distilled, gently stirred, until complete and homogenous swelling. To evaluate the effect of sterilization

process on the viscoelastic parameters, the oscillation tests were repeated on sterile samples, which were obtained by autoclaving at the condition of 121°C for 15 min.

### Monosodium iodoacetate induced arthritis (MIA) model

Monosodium iodoacetate was dissolved in saline to induce arthritis in animals. Under anesthesia by isoflurane inhalation and intraperitoneal injection of tribromoethanol, the right knee joint received a single intra-articular injection of MIA in 0.3 mg/50  $\mu$ L of sterile saline. The left knee joint also received an injection of saline. The knee fixed at 90° and MIA or phosphate buffer saline (PBS) was injected through patellar tendon (Semenzato et al., 2015). The force exerted by each limb was measured dual channel weight averager (Harvard Apparatus, Holliston, USA) and joint pain was assessed by the distribution changes of weight between right and left hind paw (right/left paw weight ratio) (Bove et al., 2003).

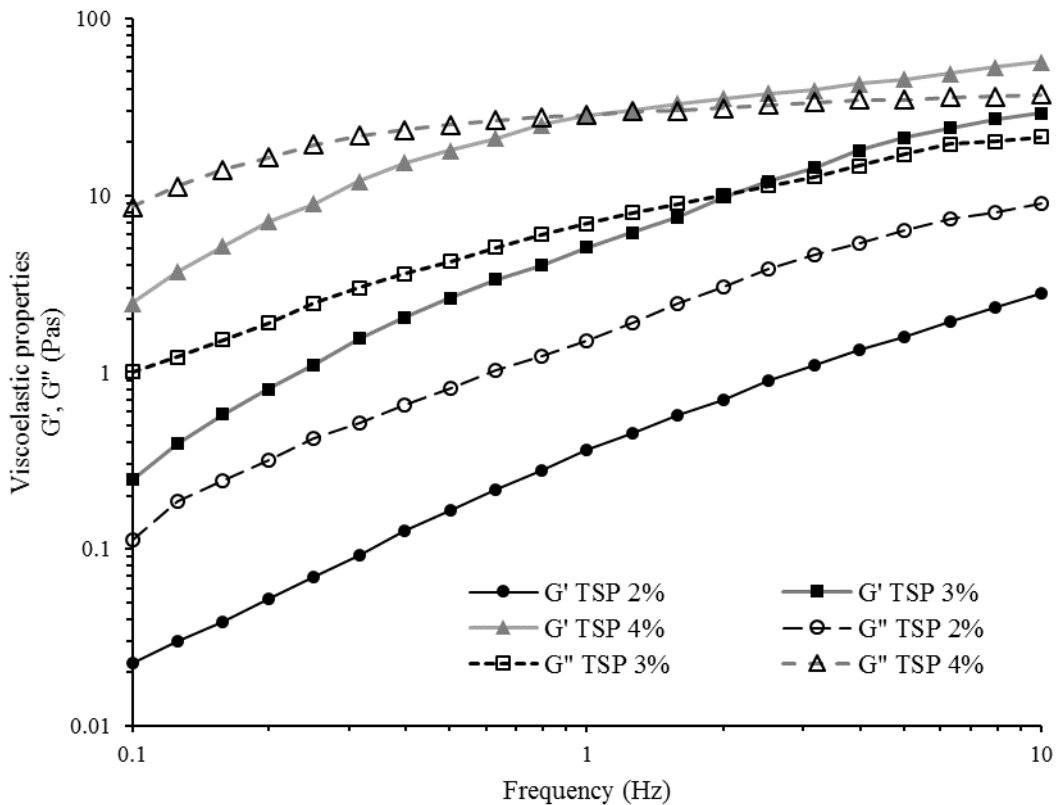
### Statistical analysis

Data were presented as means ± SE for the indicated number of independently performed experiments. Statistical significance ( $p < 0.05$ ) was assessed by one-way analysis of variance (ANOVA) coupled with Dunnett's  $t$ -tests.

## RESULTS AND DISCUSSION

### Viscoelastic properties of TSP solutions

First, the rheological properties of TSP solutions for the formulation have been measured under oscillatory flow condition at different concentrations of 2, 3 and 4% w/w. The viscoelastic properties showed a sol-gel transition as an increase of TSP concentration (Figure 1). 2% TSP showed a liquid-like behavior, that is, the loss modulus  $G''$  (elastic property) was greater than the storage modulus  $G'$  (viscous property) in all the frequency ranges and both moduli were significantly dependent on frequency. By increasing the concentration, storage moduli  $G'$  indicating the elastic character of any material was increased and became higher than the loss moduli except 2% TSP, when high frequencies were applied. The mechanical spectra of 3 and 4% TSP showed crossover point-frequency values at 1.2 and 1 Hz, respectively. As expected, the frequency value corresponding to the crossover point, which exhibited the change in rheological characteristics, decreased by increasing the concentration. These rheological results are shown to be in line with the publication by Semenzato et al. (2015).



**Figure 1:** Viscoelastic properties (storage modulus,  $G'$  and loss modulus,  $G''$ ) of TSP solution on various ratios.

### Viscoelastic properties of the formulation of HA and TSP before the sterilization

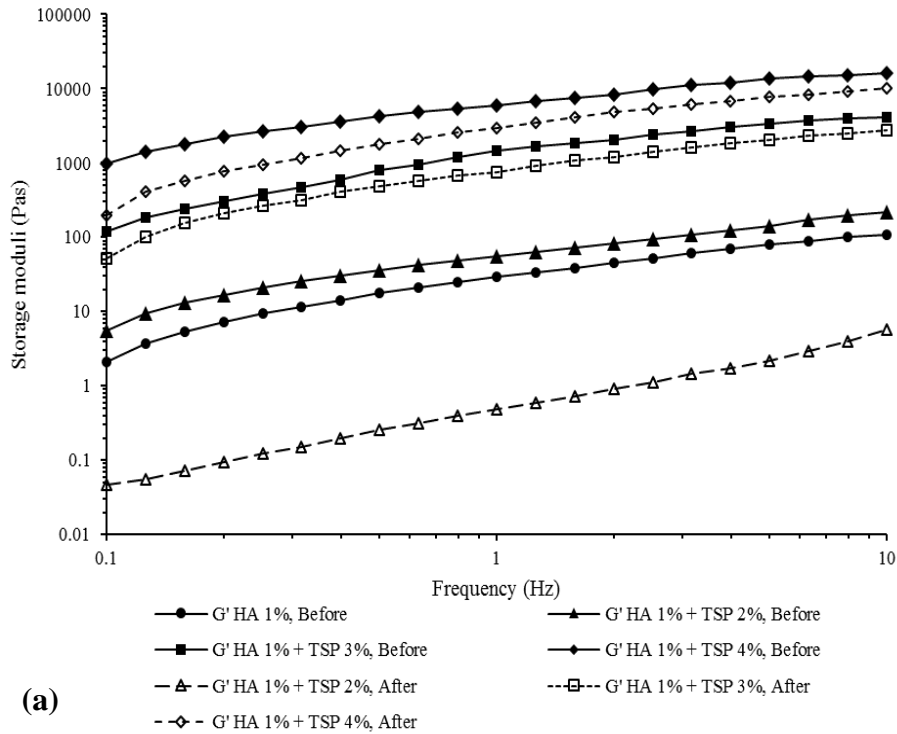
The viscoelastic properties, the dependence of the elastic ( $G'$ ) and the viscous ( $G''$ ) moduli upon the oscillation frequency, for the formulation of HA and TSP are shown in [Figure 2](#). The storage modulus of each formulation that was added with over 3 and 4% TSP, before the sterilization, is over 5 orders of magnitude higher than 2% TSP added formulation and HA alone at the all frequency ranges. The storage modulus spectra of the formulation, before the sterilization, showed a significant increase with the addition of TSP on the dose dependent manner. Adding with increased TPS showed the typical elastic properties with increasing frequency, such as a polymer. As expected, after the sterilization, storage moduli of all formulations were decreased and the pattern of rheological properties was lost with increase in frequency when compared with formulation before sterilization. As regards HA alone, it was not obtained the data of storage modulus, cause of the demolition of the molecule network by the sterilization. However, the formulation with TSP showed the increase of storage modulus on the dose dependent manner, even though it is lower than before the sterilization ([Figure 2a](#)).

The loss moduli of all formulations, before the

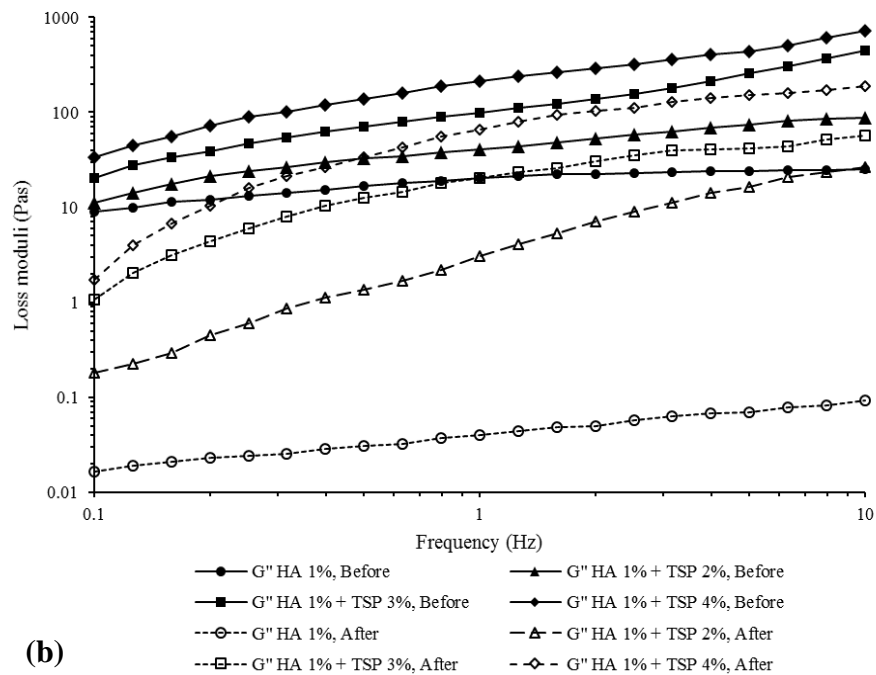
sterilization, showed the increased spectra on the increase of TSP concentration. The loss moduli of formulations added with over 3% TSP was over 2 times higher than 2% TSP added formulation at the frequency range. By adding TSP, the loss moduli of formulation were over 1.2 times higher than HA formulation alone. On the other hand, the loss moduli of all formulations was decreased significantly by the sterilization. As regards HA formulation alone, the loss modulus was decreased from 9.022 to 0.016 Pas when compared with before and after sterilization. However, the addition of TSP in HA formulation showed the resistance against decreasing loss modulus ([Figure 2b](#)).

### Viscoelastic properties of the formulation of HA and TSP after sterilization

Before sterilization, the storage modulus of HA formulation alone was lower than the loss modulus until under 0.45 Hz of the frequency and the crossover point frequency was 0.45 Hz. HA formulation alone showed elastic property, indicating that the storage modulus was higher than the loss modulus with over 0.45 Hz. By adding 2% TSP, the storage and loss modulus increased and showed similar pattern with HA formulation alone. In addition, it clearly



(a)

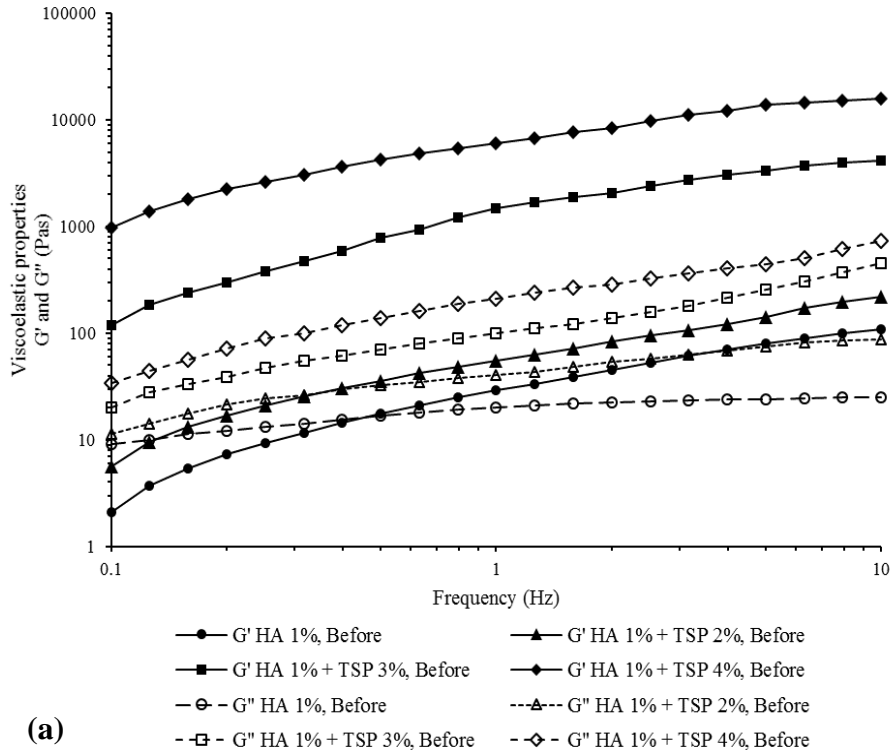


(b)

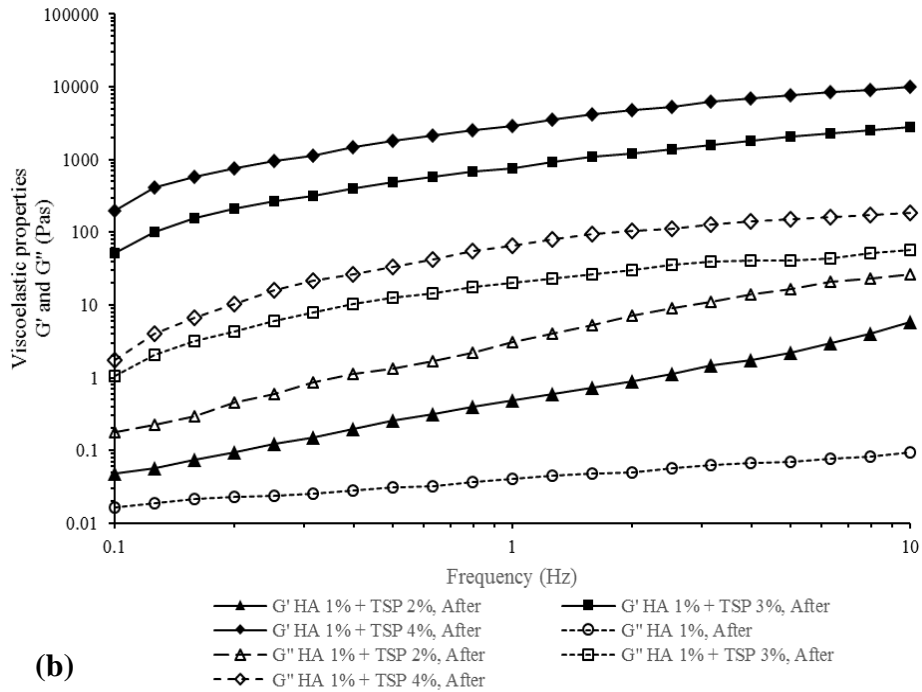
**Figure 2:** Viscoelastic properties (storage modulus,  $G'$  and loss modulus,  $G''$ ) of the formulation before the sterilization with HA and TSP on various ratios.

moved the crossover point frequency to lower range (0.33 Hz) than HA formulation alone (0.45 Hz). In the case of 3 and 4% TSP addition, their storage modulus was higher than the loss modulus at all ranges of frequency. The pattern of the viscoelastic property of 3% TSP formulation,

typical of structured materials, such as gels, is known as "weak gel behavior" (Bove et al., 2003). However, the pattern of viscoelastic property of 4% TSP formulation showed the "strong gel behavior" because the storing modulus (984.9 Pas) is over two order of magnitude higher



(a)



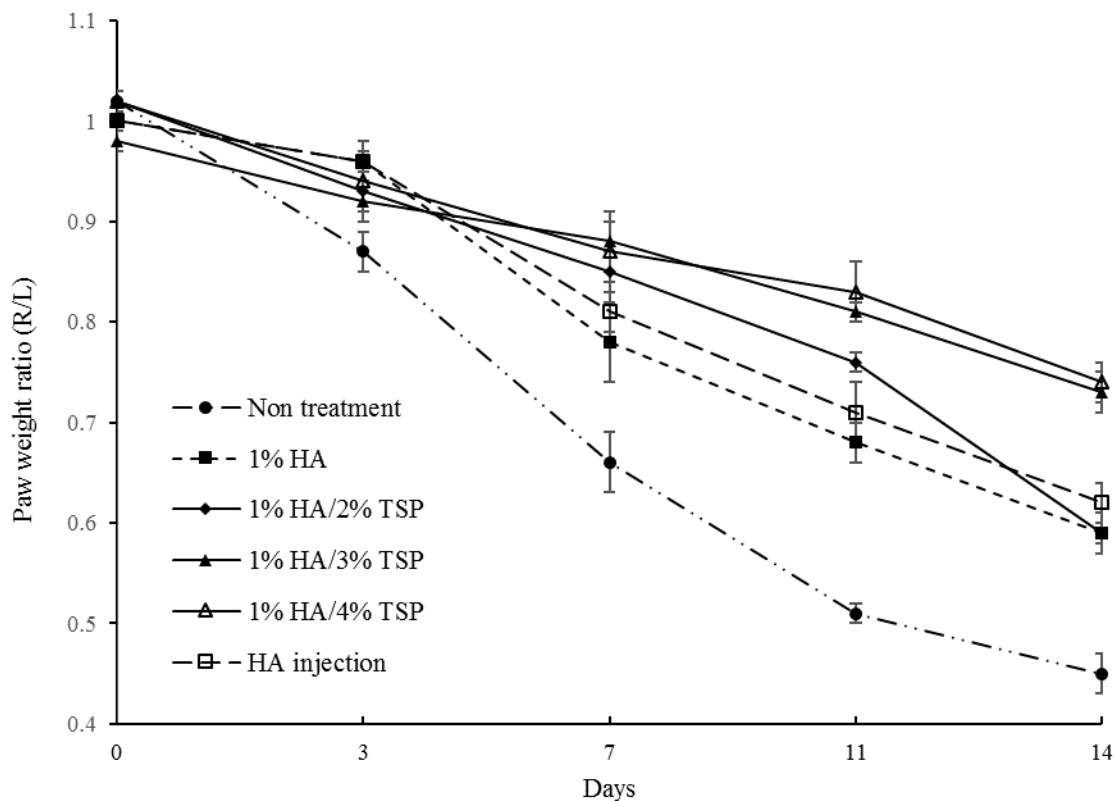
(b)

**Figure 3:** Viscoelastic properties (storage modulus,  $G'$  and loss modulus,  $G''$ ) of the formulation After the sterilization with HA and TSP on various ratios.

than the loss modulus (33.7 Pas) at the frequency of 0.1 Hz (Figure 3a).

After sterilization, the storage modulus of 2% TSP formulation was lower than the loss modulus in the range

of all frequencies. Heat sterilization resulted in the reduction of some elastic characters by the deformation of molecular network in each formulation, even though it seems to give a resistance against the heat. Over 3%



**Figure 4:** Lubricant effect of the formulations with various concentrations of TSP using monosodium iodoacetate induced arthritis model. All weight-distribution data are expressed as mean±SE (n=6).

formulations showed that the storage modulus was higher than the loss modulus in the range of all frequencies. By over 3% TSP addition in the formulation, it clearly protected the reduction of elastic property against the heat (Figure 3b).

### Analgesic effect of the formulations

The effect of various formulations on the monosodium iodoacetate-induced arthritis in rats is shown in Figure 4. Induction of arthritis by monosodium iodoacetate is optimally turned up on all experimental groups. The paw weight ratio of non-treatment group as negative control decreased by significant scores from day 3 to day 14. HA injection as positive control showed its analgesic effect on arthritis induced rats, from day 3 to 14. It improved the scores of paw weight ratio,  $0.96 \pm 0.02$ ,  $0.81 \pm 0.03$ ,  $0.71 \pm 0.03$  and  $0.62 \pm 0.02$  when compared with the scores of the non-treatment group,  $0.87 \pm 0.02$ ,  $0.66 \pm 0.03$ ,  $0.51 \pm 0.01$  and  $0.45 \pm 0.02$ . It was a very similar effect to 1% HA formulation, that is, the same dose with HA injection. The formulation added with 2% TSP showed similar effect on change in paw weight ratio and lower effect than HA injection on the day 14.

Each formulation with 3 and 4% TSP significantly

improved the right/left weight ratios,  $0.73 \pm 0.02$  and  $0.74 \pm 0.02$ , respectively when compared with the ratio of right/left paw from  $0.45 \pm 0.02$  as the non-treated group at the day 14. The results obtained in this study clearly showed the protective effect of TSP with HA formulation on the arthritis animal model induced with monosodium iodoacetate.

### CONCLUSIONS

The investigation of the rheological properties of polysaccharide aqueous dispersions represents a powerful tool to optimize their use in the injection for arthritis. Hyaluronic acid and Tamarind seed polysaccharide, with a similar molecular weight, showed very close rheological properties when used in the concentration range of 2 - 4%, both under continuous and oscillatory flow conditions (Semenzato et al., 2015). Tamarind seed polysaccharide is a potential material used as a substitute for hyaluronic acid or a combination with hyaluronic acid in injection for arthritis without any toxic chemical reaction with some cross-linking agents such as 1,4-Butanediol diglycidyl ether, Divinyl Sulfone and 1-ethyl-3-[3-(dimethylamino) propyl] carbodiimide etc. In the future, a further study is required to determine the effect and safety they have on arthritis

model between the cross-linked HA gels and HA formulation with TSP.

## ACKNOWLEDGMENT

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