Indian Journal of Traditional Knowledge
Vol 18(4), October 2019, pp 818-823

Anti-inflammatory effect of traditional herbal formula Jonlon-5 decoction in an animal model of inflammation

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Received 10 July 2018; revised 27 June 2019

The study is an attempt to assess the effects of a traditional Mongolian medicine, Jonlon-5, against inflammation in an animal model. Both acute and sub-acute toxicity of Jonlon-5 were evaluated. The level of acute toxicity of Jonlon-5 was determined in accordance with the current guidelines by Organization for Economic Co-operation and Development (OECD). A sub-acute toxicity study was done by oral administration of Jonlon-5 with doses of 100, 200, and 500 mg/kg body weight/daily for 28 days. Paw edema was produced by a sub-plantar injection of 1% carrageenan. Biochemical and immunological analyses were performed at the end of the study. No adverse effects of Jonlon-5 were observed even at doses up to 2000 mg/kg/day. In the sub-acute study, there was a significant increase ($p<0.05$) in the activity of serum liver enzymes of rats, administered with 500 mg/kg body weight of Jonlon-5. The results demonstrate that Jonlon-5 can significantly reduce paw edema and inhibit the production of malondialdehyde (MDA), tumor necrosis factor-α and interleukin-1β ($p<0.05$). Our findings suggest that Jonlon-5 has anti-inflammatory effects in carrageenan-induced in rats with arthritis. However, intake of high doses may exhibit mild liver toxicity.

Keywords: Acute inflammation, Acute toxicity, Carrageenan, Jonlon-5, Traditional medicine

IPC Code: Int. Cl.[19]: A01N 25/32, A61K 31/731, A61K 36/00

The number of people with arthritis is rising rapidly around the world[1,2]. Arthritis is one of the leading causes of disability among adults and is very costly in both personal and economic terms[2]. Despite many advances in the management of arthritis, progress on finding a cure remains elusive. This is because currently available anti-arthritis medications have adverse effects with long-term use, creating the need for more effective and safe treatment options. An alternative with some advantages, such as fewer side effects and lower cost, is herbal medicine[3].

In traditional Mongolian medicine, Jonlon-5, a mixed herbal medicine, is used to treat fever, infection, arthritis and heart disease[4]. Jonlon-5 tastes bitter and has a cool potency. It is composed of five herbs: Radix *Sophoroe alopecuroides*, Herba *Gentianaebar batae*, Fructus *Gardeniae jasminoides*, Fructus *Terminaliae chebulae* and Fructus *Terminaliae belliricae*. Previous phytochemical studies have revealed that Jonlon-5 contains alkaloids (mainly matrine and oxymatrine), flavonoids, saponin, and organic oxides[5].

S. alopecuroides. L (Unegen suulkhei lider), the main compound in Jonlon-5, is rich in alkaloids and flavonoids and has anti-inflammatory, anti-oxidative and anti-bacterial effects[6,7]. *G. barbatae* (Sormuust degd) and its main active alkaloids, flavonoids, xanthone, and secoridoids have been found to have anti-inflammatory, hepato-protective, and anti-oxidative effects[7-10]. *G. Jasminoides* (Arur), containing geniposide, genipin and crocetin has a broad spectrum of therapeutic effects that include anti-oxidative, anti-inflammatory, anti-thrombosis and anti-atherosclerosis activities[11]. Evidence from previous pharmacological and ethno-medical studies have shown that *T. Chebula* (Jurur) exhibits anti-bacterial, anti-inflammatory, immune-modulatory, hypoglycemic, anti-oxidative and analgesic effects and confers hepatic protection[12,13]. *T. bellirica* (Barur) has been reported to have analgesic, anti-inflammatory, anti-secretory and hepato-protective activities[14,15]. The latter 02 plants are rich in gallic acid, which has strong anti-oxidative activity in human tissues[16].

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The traditional approach of combining several herbal medicines has been found to be more effective than administering them in isolation[17]. Given evidence that Jonlon-5 and its herbal components have potential anti-bacterial, anti-inflammatory and anti-oxidative activities, we hypothesized that Jonlon-5 could be a safe and effective medication against inflammation. In the present study, we investigated the anti-inflammatory activity of Jonlon-5 on animals that were induced with paw edema. The acute and sub-acute toxicity of Jonlon-5 were also evaluated.

Material and methods

Preparation of Jonlon-5

Jonlon-5 herbal medicine was prepared in the Institute of Traditional Medicine and Technology of Mongolia (Table 1). The crude components of Jonlon-5 were authenticated by experts from Department of Botany, Institute of Biology, Mongolia. One gram of crushed dried plant material was dissolved into 100 mL of distilled or deionized water and boiled at 100°C for 15 min. The prepared solution was used for the tests.

Experiment animals

The current study was performed on Wistar albino rats of both sexes weighting 200 to 250 g at the pharmacology laboratory of the Institute of Traditional Medicine and Technology of Mongolia. The animals were kept and followed at standard laboratory housing conditions (12 h/12 h light/dark cycle). They were fed with pellets and sterilized tap water.

For the acute study, healthy white mice of both sexes weighting 22–28 g were taken from the animal house attached to the Institute of Traditional Medicine and Technology of Mongolia and used after one week of quarantine. All experiments were carried out in accordance with the institutional guideline for the care and use of laboratory animals. The ethical approval was sought from the Ethics Committee of the Mongolian National University of Medical Sciences.

Acute toxicity test

In animals, a single oral administration of Jonlon-5 showed no toxicity at a dose of up to 2000 mg/kg. Accordingly, a dose of 2000 mg per kg was used as the maximum dose as recommended by the current guidelines of OECD. Thirty mice of each sex were randomly placed to two groups with fifteen mice per group. The animals in treatment group received a single dose of 2000 mg/kg via gavage. Control mice were given an equal amount of distilled water. After oral administration, all clinical signs were recorded before and after dosing at 1,2,4 and 24 h.

Sub-acute toxicity test

For the sub-acute toxicity test, 40 albino rats were randomized into 04 groups with ten rats per group. The animals in the control group were administered with 0.5 mL of saline once daily for 28 days. The animals in the other 03 groups were orally treated via oral administration of 100, 200 and 500 mg/kg of body weight of Jonlon-5, respectively, for 28 days. The rats were under constant daily observation for any signs of toxicity and their body weights were also recorded weekly throughout the experimental period. On day 29 of the experiment, all the animals in the 04 groups were anesthetized under ketamine hydrochloride (80 mg/kg). Blood samples or clots were collected by cardiac puncture for biochemical investigation and centrifuged according to groups. The serum was separated into sterile bottles for biochemical analysis.

Carrageenan-induced inflammation

The animals were randomized into 04 groups (ten in each group): normal control (NC), inflammation, indomethacin (IMC), and Jonlon-5 (Jonlon-5 at 100 mg/kg of body weight) groups. The rats in the control and inflammation groups were administered distilled water orally (5 mL/kg of body weight). The rats in the IMC group were given

Table 1 — Crude components of Jonlon-5 (1 g)

Mongolian name	Scientific name	Used part	Amount (g)
Unegensuulkheilider	Sophora alopecuroides L.	Root	0.270
Jurur	Terminalia chebula	Fruit	0.270
Arur	Gardenia jasminoides	Herb	0.176
Barur	Terminalia bellirica	Fruit	0.168
Sormuustdegd	Gentiana barbata	Fruit	0.140
Total amount			1.00

oral indomethacin (10 mg/kg of body weight) 2 h prior to the induction of arthritis. Indomethacin has been widely used for arthritis treatment of because of its anti-inflammation and analgesic activities. The Jonlon-5 group received Jonlon-5 (100 mg/kg of body weight) 2 h prior to the induction of inflammation. Inflammation was induced by 0.1 mL 1% carrageenan. Paw volume was measured at base line, 30,60,120, and 240 min after induction with a plethysmometer. After 4 h, the rats were sacrificed, and blood was collected for various analyses.

Biochemical analysis

Determination of the level of MDA was determined using the fluorimetric method according to athiobarbituric acid test. In brief, 0.5 mL of plasma was heated in a boiling water bath for 15 min with a solution of 0.68% 2.5 mL thiobarbituric acid and followed by incubating for 15 min on ice. The mixture then was centrifuged at 12000 rpm for 5 min at 4°C. The MDA was spectro fluorometrically determined using a synchronous technique with excitation at 532 nm.

Plasma levels of cytokines

After being allowed to stand for 30 min, the blood was centrifuged at 3000 rpm/min for 10 min at 4°C; then, plasmas were collected for analysis. The serum concentrations of the tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β) were determined using a commercial enzyme-linked immunosorbent assay (ELISA) method (ELISA kit, Republic of China).

Statistical Analyses

The data are expressed as the mean ± standard deviation. A one-way ANOVA was performed to identify the difference between groups, and followed with Tukey' s test. Statistical significance was set at the level of $p<0.05$. The statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS) version 20.0 for Windows.

Results

Acute toxicity of Jonlon-5

We evaluated the acute toxicity of Jonlon-5. There were no significant differences in body weight change findings between the Jonlon-5 and NC groups, and no clinical signs were observed in the Jonlon-5 group.

Sub-acute toxicity

At the end of the experiment, there was a slight weight reduction in the Jonlon-5 treated rat groups compared to the control, but it was not statistically significant. This indicates the healthy status of the rats following Jonlon-5 decoction. Serum amino transferases (ALT and AST) and alkaline phosphatase (ALP) activity significantly increased in a dose-related manner when the rats received various doses of Jonlon-5. ALT and AST activities were significantly higher in the 500 mg/kg body weight treated group when compared with the others ($p<0.05$). ALP activity showed no significant difference compared with the others (Fig. 1).

Effects of Jonlon-5 on carrageenan-induced arthritis

The administration of carrageenan in the 03 groups led to a time dependent rise in size of paw, which reached a maximum at 4 h. Compared to the control group, pre-treatment of Jonlon-5 at a concentration of 100 mg/kg of body weight significantly ($p<0.05$) reduced the development of paw edema, as shown in Fig. 2.

Effects of Jonlon-5 on MDA concentration

The level of MDA which is a product of lipid peroxidation, was significantly elevated in the inflammation group as compared those in the NC group. However, the IMC group exhibited a significant fall in MDA levels compared with the inflammation group. In addition, the Jonlon-5 group have shown a significant decrease in the level of MDA ($p<0.05$) compared to other groups (Fig. 3).

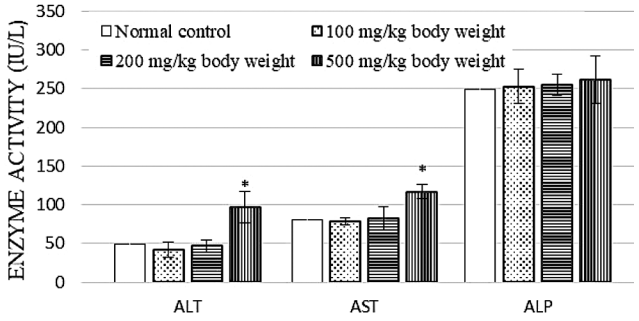

Fig. 1 — Effect of Jonlon-5 on the activity of serum liver enzymes. Normal control group: n=10; Jonlon-5 100 mg/kg body weight group: n=10; Jonlon-5 200 mg/kg body weight group: n=10; Jonlon-5 500 mg/kg body weight group n=10. Data are reported as mean ± SD. Data with * are significantly different ($p<0.05$)

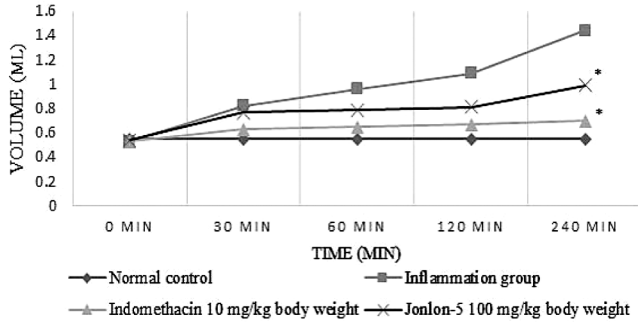

Fig. 2 — Effect of Jonlon-5 on Carrageenan-induced rat paw edemaNormal control group: n=10; Inflammation group n=10; Indomethacin 10 mg/kg body weight group: n=10; Jonlon-5 100 mg/kg body weight group: n=10; Data with * are significantly different ($p<0.05$)

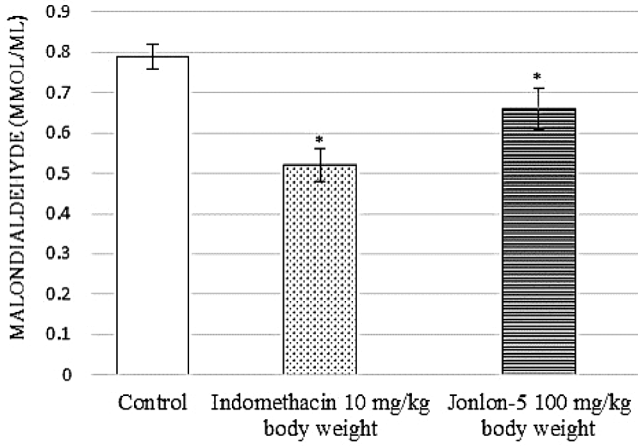

Fig. 3 — Effect of Jonlon-5 on the level of malondialdehyde (MDA). Control (inflammation) group: n=10; Indomethacin 10 mg/kg body weight group: n=10; Jonlon-5 100 mg/kg body weight group: n=10. Data are reported as mean ± SD. Data with * are significantly different ($p<0.05$).

Effects of Jonlon-5 on the release of pro-inflammatory cytokines of plasma

The in vivo anti-inflammatory activity of Jonlon-5 was also examined by assessing the levels of TNF-α and IL-1β by ELISA. As Fig. 4 presents, the TNF-α and IL-1β levels elevated significantly in plasma after carrageenan-induced inflammation compared with the NC group. However, pre-treatment with Jonlon-5 (100 mg/kg of bodyweight) decreased TNF-α and IL-1β productions in plasma and was significantly different compared to other groups ($p<0.05$). Interestingly, Jonlon-5 at 100 mg/kg of bodyweight significantly suppressed the release of cytokines including TNF-α and IL-1β, which was clearly comparable to the effect of the non-steroid anti-inflammatory medication In domethac in (10 mg/kg of body weight).

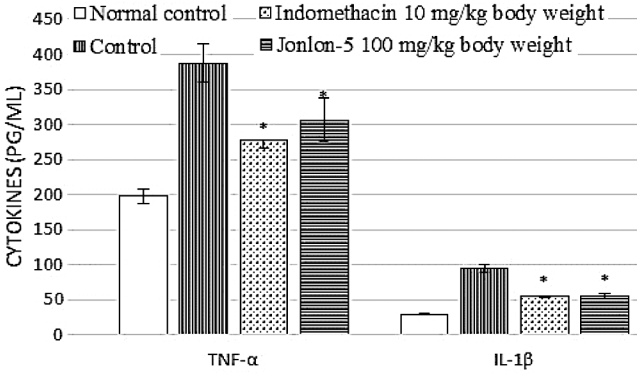

Fig. 4 — Effect of Jonlon-5 on the expression of tumor necrosis factor (TNF-α) and interleukin (IL-1β) protein in plasma. Normal control group: n=10; Indomethacin 10 mg/kg body weight group: n=10; Jonlon-5 100 mg/kg body weight group: n=10. Data are reported as mean ± SD. Data with * are significantly different ($p<0.05$)

Discussion and conclusion

In traditional medicine, combinations of multiple herbs are used to increase their pharmacological activity and reduce adverse reactions[17]. Thus, in this study, we focused on whether the combination of herbs in Jonlon-5 has anti-inflammatory and anti-oxidative activities that are associated with a reduction in oxidative stress and inflammation and whether it can be used safely.

The acute toxicity study allowed us to determine the safety of the materials. The study indicated that Jonlon-5 is a safe agent when given orally to rats in a single dose at a level of 2000 mg/kg. Moreover, no clinical signs of adverse events were observed. According to the Guidance on Acute Oral Toxicity Testing based on an oral LD50 value that was recommended by the OECD, Jonlon-5 may be assigned as class 5 (LD50>2000 mg/kg body weight), which was appeared to be the low toxicity class[18].

Administration of Jonlon-5 at two different doses (100 mg/kg and 200 kg/mg body weight) for 28 days had no statistically significant effect on serum levels of ALT and AST. The current study showed that Jonlon-5 up to 200 mg/kg body weight did not indicate any toxicity in the liver for the parameters used, ALT and AST. However, serum ALT and AST levels were found to increase significantly in rats receiving 500 mg/kg body weight of Jonlon-5 when compared to the control group. The significant increase in ALT and AST suggests that administration of high doses of Jonlon-5 may induce destruction of liver cells.

Carrageenan is often used to produce non-immune-mediated inflammation when evaluating components with potential anti-inflammatory activity. In the current study, anti-inflammatory activity of Jonlon-5 is observed 30 min after carrageenan induction compared to the inflammation group. Our results demonstrate that Jonlon-5 has an anti-inflammatory effect via reduction of paw edema that is associated with carrageenan induction. In line with these observed results, past studies have shown that the administration of Sendeng-4 (Fructus *Terminaliae chebulae*, Fructus *Terminaliae belliricae*, Fructus *Gardenia jasminoides,* and Fructus *Toosendan*), and Lider-7 (Radix *Sophoroe alopecuroides*, Radix *Inulae helenium*, Fructus *Gardeniae*, Fructus *Terminalia belliricae*, Fructus *T. chebulae*, Herba *Gentiana barbatae,* and Herba *Lagotis integrifoliae*), which have similar components asJonlon-5, exhibited an anti-inflammatory effect on both acute and chronic experimental inflammation[19-21].

According to the literature, carrageenan induced inflammation is associated with free radicals that lead to elevated lipid peroxidation[6]. MDA, a product of lipid per oxidation, can be used as a biomarker of oxidative stress in tissues and plasmas. In the current study, we found that Jonlon-5 attenuated the rise in MDA level in plasma, indicating that Jonlon-5 can weaken the process of lipid per oxidation implicated in the pathogenesis of carrageenan induced inflammation.

TNF-α and IL-1β are inflammatory cytokines that are released during inflammation process and play a significant role in defenses of organisms. Several studies have demonstrated an inhibition effect of various traditional medical treatments on the levels of TNF-*α* and IL-1β. *Wen et al.* reported that traditional Chinese herbs inhibited the release of inflammatory cytokines as well as levels of nitric oxide synthase and cyclooxygenase-2 in a carrageenan-induced paw edema[22]. Similarly, our study showed that pre-treatment with Jonlon-5 reduced levels of TNF- and IL-1β cytokines in plasma.

Our study has some limitations to note. There was no histological examination to confirm the therapeutic effect of Jonlon-5 in an experimental model of arthritis. Another limitation of the study is the inability to include various doses and hence to determine the dose-dependent anti-inflammatory effect. This would have made the study more comprehensive, although the safe dose the authors chose provided a positive result.

In conclusion, our findings suggest that Jonlon-5 is a safe drug with some anti-inflammatory effect in an acute model. It was revealed that Jonlon-5 can suppress the release of MDA, IL-6 and TNF-α in plasma. Jonlon-5 is a promising anti-inflammatory agent against inflammation but can cause mild liver damage at high doses.

Acknowledgements

The authors are very grateful to all staffs of the pharmacology laboratory and animal house, the Institute of Traditional Medicine and Technology, Mongolia, for valuable contribution and support to the successful completion of the study and Mr Turner for language editing.

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