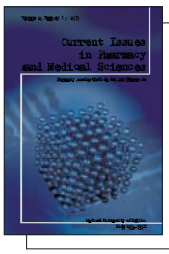

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Ànalgesic and anti-inflammatory activities of traditional mongolian drug Garidi-5

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ABSTRACT

Garidi-5, a traditional medicine composed of 5 herbs including Terminalia chebula Retz., Aconitum Kusnezoffii Reichb., Acorus calamus L., Saussurea lappa L., and musk of Moschus moschiferus, has been used in traditional Mongolian medicine as an analgesic and antibacterial medicine. The present work was undertaken to evaluate the traditional drug Garidi-5 for its anti-inflammatory activity. **Methods**. The method of Winter et al. was used to study acute inflammation. Rats in groups of five each were treated with vehicle, Garidi-5 (20, 80 and 200 mg/kg, p.o.) and Indometacin (10 mg/kg) one hour prior to Carrageenan injection. 0.1 ml of 1% Carrageenan was injected into the subplantar tissue of left hind paw of each rat. Swelling of carrageenan injected foot was measured at 0, 0.5, 2, 4 h using Plethysmometer (UGO Basile, Italy)). The right hind paw was injected with 0.1 ml of vehicle. **Results**. The Garidi-5 (20, 80 and 200 mg/kg) significantly ($P<0.01$) inhibited carrageenan induced rat paw edema as compared to control group. Maximum inhibition of paw edema was observed with Garidi-5 (80 and 200 mg/kg) at 4 h when compared to the control group. In assay data, the TNF-α secretion in serum were highly elevated by carrageenan induction but administration of Garidi-5 significantly reduced serum secretion of inflammatory mediators as compared to vehicle group. **Conclusion**. In conclusion, these results suggested that Mongolian traditional drug Garidi-5 analgesic and anti-inflammatory effects.

Keywords: Garidi-5, Anti-inflammatory; Carrageenan

INTRODUCTION

Garidi-5 has been used in traditional Mongolian medicine as an antibacterial and analgesic agent for treatments of various diseases including typhus, dyphteria, joint conditions, neurological and skin disorders. Garidi-5 is composed of 5 herbs including *Terminalia chebula* Retz., *Radix Aconitum Kusnezoffii* Reichb., *Acorus calamus* L., *Saussurea lappa* L., and musk of *Moschus moschiferus* [4].

Some of its constituents like Radix Aconitum kusnezoffii (Mei Chou Lai et al., 2011) [10], A. calamus (Gacche et al., 2006) [6], T. chebula (Shin TY et al., 2001) [15] were claimed to reduce inflammation and pain.

The major components of Radix Aconiti Kusnezoffii are alkaloids, such as aconitine, mesaconitine, jesaconitine, and hypaconitine, as well as non-alkaloid constituents such as sucrose, daucosterin, meso-inositol, and higher fatty acids [10].

T. chebula, though, contains several phytoconstituents like tannins, flavonoids, sterols, amino acids, fructose, resin, anthraquinones, ethaedioic acid, sennoside, 4,2,4 chebulyl-d-glucopyranose, terpinenes and terpinenols have also been reported to be present [13,16]. Triterpenoids and their glycosides have been isolated from stem bark of *T. ch.ebula* [8].

Calamus (as various extracts of the rhizome) contains constituents such as alkaloids, flavonoids, gums, lectins, mucilage, phenols, quinone, saponins, sugars, tannins, and triterpenes (steroids) [1, 2,11].

Inflammation is a response to tissue damage that involves enzyme activation, cytokine release, organ swelling, cell migration, and tissue repair [17].

The release of free radicals from damaged tissues has been demonstrated to play a very important role during inflammation, and can be identified within the first six hours after λ-carrageenan administration, along with elevated levels of prostaglandins and nitric-oxide. Several studies also indicated that inflammation can stimulate ma-

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londialdehyde (MDA) production in response to cell membrane damage arising from free radicals [9].

The present work was undertaken to evaluate the traditional drug Garidi-5 for its anti-inflammatory activity [19].

MATERIALS AND METHODS

Animals. Specific pathogen-free white wistar rats (180-220 g) were used for the study and all were housed in a quiet room with 12 h light/dark cycle. The study protocol was approved by the Ethical Committee of the Health Sciences University of Mongolia and the care and handling of animals were in accordance with the principles of the Helsinki *Declaration*.

Material. Garidi-5 produced in the Traditional Drug Manufacture of Traditional Medical Science, Technology and Production Corporation of Mongolia, was used in this study.

Acetic acid-induced writhing test. Swiss albino mice were divided into four groups (n = 6). Group I received acetic acid (0.6% v/v, 10 ml/kg b.w., i.p.) and writhing reflex was noted for the period of 20 minutes. Group II received aspirin (Acetyl salicylic acid) 100 mg/kg b.w. p.o. Group III, IV and V received Garidi-5 at the doses of 20mg/kg, 80 mg/kg and 200 mg/kg b.w., p.o. respectively. 1h min after aspirin and Garidi-5 administration, group I-V received acetic acid (1% v/v, 10 ml/kg b.w., i.p.) and writhing reflex was noted for the period of 20 min.

Carrageenan induced rat paw edema. The rats were divided into four groups containing six rats in each group. 0.1 ml of 1.0% carrageenan in normal saline (0.9% w/v NaCl) was injected to the sub plantar region of right hind paw. The Garidi-5 was administered to the rats 1 h before carrageenan injection. Different groups were treated as follows:

- Group I: Carrageenan (0.1 ml of 1.0% carrageenan/rat to the sub plantar region).
- Group II: Carrageenan + Indomethacin (10 mg/kg b. w., p.o.)
- Group III-V: Carrageenan + Garidi-5 (20mg/kg, 80 mg/kg and 200 mg/kg b. w., p. o. respectively). (Winter et al. 1962

Swelling of carrageenan injected foot was measured at 0, 0.5, 1, 2, 4 h using Plethysmometer (UGO Basile, Italy) (Vogel, 2002).

Blood samples were withdrawn after the 4th hour of carrageenan induction, centrifuged at 3500 rpm. Sera aliquots were frozen at-80°C for analysis of TNF-α.

RESULTS

Effect of Garidi-5 on acetic acid-induced writhing in mice

Garidi-5 (20 mg/kg, 80 mg/kg, and 200 mg/kg) significantly (p.05) reduced abdominal writhes induced by acetic acid in mice by 40.4-47.9% (Fig. 1). This result suggests that the water extract of Garidi-5 possesses significant peripheral analgesic effect.

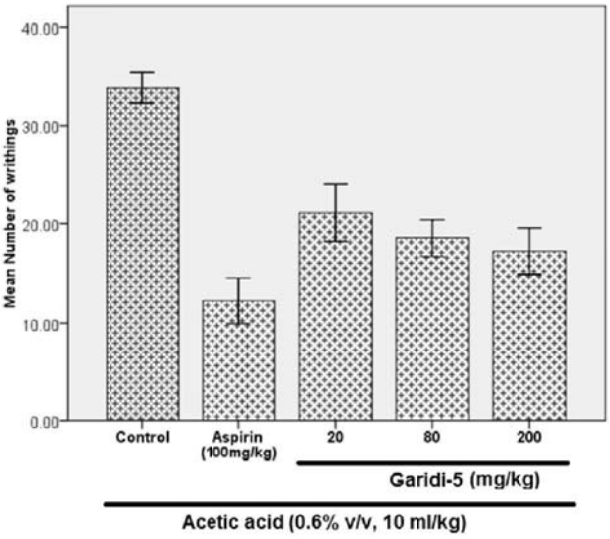

Fig. 1. The analgesic effects of Garidi-5 on acetic acid-induced writhing in mice

Carrageenan induced rat paw edema

The Garidi-5 (20, 80 and 200 mg/kg) significantly ($P < 0.05$) inhibited carrageenan induced rat paw edema as compared to control group. Maximum inhibition of paw edema was observed with Garidi-5 (200 mg/kg) at 4 h when compared to the control group. Indomethacin inhibited paw edema by 49.54%. The observations are given in Table 1.

In assay data, the TNF-á secretion in serum were highly elevated by carrageenan induction but administration of *Garidi-5* (20, 80, 200 mg/kg) significantly reduced serum secretion of inflammatory mediators as compared to vehicle group (Fig. 2).

TNF-α secretion was magnified by carrageenan injection (367.81 pg/ml). TNF-á secretion was reduced, comparable to carrageenan group, as a respond to extract administration. Administration of Garidi-5 (80, 200mg/kg)

Table 1. Anti-inflammatory effect of Garidi-5 on carrageenan induced rat paw oedema

Treatment (mg/kg)	Mean increase in paw volume (ml)				
	0 h	0.5 h	1h	2h	4 h
Control	0.51± 0.039	0.88± 0.051	1.07± 0.083	1.02± 0.079	1.65± 0.094
Indometacin (10)	0.50± 0.015	0.61± 0.035*	0.60± 0.039*	0.55± 0.041*	0.52± 0.032
Garidi-5 (20)	0.52± 0.049	0.79± 0.012*	0.76± 0.027*	0.70± 0.055*	0.68± 0.021*
Garidi-5 (80)	0.53± 0.032	0.71± 0.031*	0.69± 0.047*	0.65± 0.026*	0.59± 0.054
Garidi-5 (200)	0.51± 0.061	0.70± 0.057*	0.67± 0.069*	0.64± 0.054*	0.57± 0.061

n = 10. The observations are mean ± S.E.M. *P< 0.05, as compared to control.

significantly reduced inflammatory mediator (TNF-á) secretion by 21.5% - 22.5% to be close to inhibition level of indomethacin administration (28.3%).

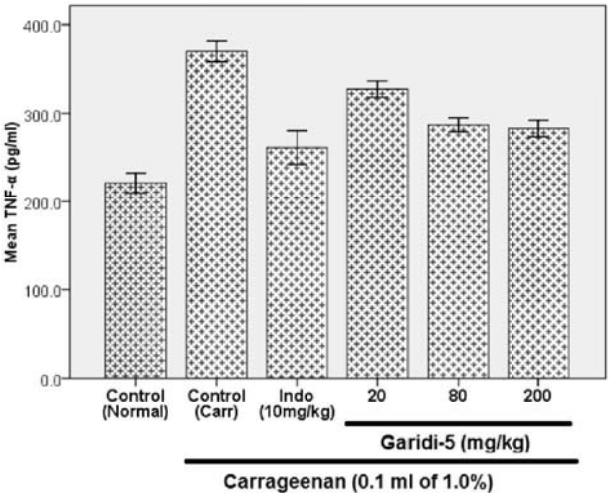

Fig 2. Effect of *Garidi-5* on serum inflammatory cytokines secretion in carrageenan induced paw oedema in rat

DISCUSSION

The present study evaluated the analgesic and anti-inflammatory activities of Garidi-5 in experimental rodent models. The results are presented in Tab 1., Fig 1-2. The acetic acid induced writhing test is normally used to evaluate the peripheral analgesic effect of drugs and chemicals. The response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathway.

The model is based on pain emanating from internal organs, which is mediated through prostaglandins generated from arachidonic by the enzyme cyclooxygenase. Increased prostaglandin levels, particularly PGE2á, can be detected quantitatively in peritoneal fluid after acetic acid injection using radioimmunoassay. Thus, prostaglandin production plays an important role in the pain response. This study showed that Radix Aconiti Kusnezoffii is able to produce a dose-dependent reduction in acetic acid-induced writhing, to a degree comparable to indomethacin. In addition, the analgesic effect might be associated with the inhibition of arachidonic acid metabolism.[5]

The aconitine-alkaloids aconitine, mesaconitine and hypaconitine were used as standards in analyzing the components of Radix Aconiti Kusnezoffii. Among these alkaloids, mesaconitine was found to be the most abundant constituent of Radix Aconiti Kusnezoffii, followed by aconitine and then hypaconitine. In the acetic acid-induced writhing test, mesaconitine and aconitine exhibited marked analgesic effects, producing significant reductions in body writhing.[7]

The petroleum ether, chloroform, ethanol and water extracts of *T. chebula* fruits were evaluated for their analgesic activity using the tail immersion model in mice. The ethanolic extract of the plant exhibited analgesic response at 200,400 and 800 mg/kg body weight in acute pain and in chronic pain studied for 15 days with maximum analgesic response on 14th day. The results suggested that *T. chebula* could be a potential candidate for bioactivity-guided isolation of natural analgesic agents in the management of chronic pain.[12]

Oral administration of Garidi-5 significantly reduced the number of writhings induced by acetic acid in mice (Fig. 1) and the activity was comparable to that observed with 100 mg/kg Aspirin (used as a reference drug, po). Figure 1 shows that Garidi-5 inhibited acetic acid-induced abdominal constrictions in mice, thereby exhibiting an antinociceptive effect.

A common animal model for the investigation of anti-inflammatory drugs is ë-carrageenan injection into the foot of a mouse.[18] This triggers increased permeability of local blood vessels, resulting in foot swelling. This method is often employed to evaluate the efficacy of anti-inflammatory agents and the anti-edema effects of natural products.

The response to the injection of ë-carrageenan-induced paw edema is biphasic, and the injected ë-carrageenan releases various substances with time to induce inflammation. In phase I, within 1 to 2 h of λ-carrageenan injection, histamine, serotonin and other mediators are released. During phase II, i. e., within 3 to 5 h of injection, prostaglandin is released. Between the two phases, the substances like kinin are released.[3]

TNF-*á* is a major mediator in inflammatory responses, inducing innate immune responses by activating T cells and macrophages and stimulating secretion of other inflammatory cytokines [14]. Also, TNF-*á* is a mediator of Carrinduced inflammatory incapacitation and is able to induce the further release of kinins and leukotrienes, which is suggested to have an important role in the maintenance of long-lasting nociceptive response.

There are also studies showing that ë-carrageenan-induced inflammation is associated with free radicals. Radix Aconiti Kusnezoffii was shown to exert anti-inflammatory activity in both phases of the response, especially at the dose of 40 mg/kg, which presented the best efficacy. It can therefore be deduced that the anti-inflammatory action of Radix Aconiti Kusnezoffii can be attributed to inhibiting the release of the early phase mediators, such as histamine and serotonin, and the late-phase substance prostaglandin.

We also evaluated the anti-inflammatory effects of Garidi-5 on paw edema induced by Carr in mice and detected the levels of TNF-á the paw edema 4 h after Carrageenan injection.

It is clear from Table 1. that Garidi-5 produced a dose-dependent inhibition of Carrageenan induced paw edema. Statistical analysis revealed that Garidi-5 and Indometha-

cin significantly inhibited the development of edema 1–4 h after treatment and they both showed anti-inflammatory effects in Carrageenan induced rat paw edema. In the current study, the levels of TNF-α were decreased significantly in a dose dependent manner by treatment with 20, 80 and 200 mg/kg of Garidi-5.

The results of this investigation indicate that Garidi-5 exhibits an antinociceptive activity at peripheral levels and an expressive anti-inflammatory effect. Besides, care has to be taken while using Garidi-5 in the clinical administration due to it's lower therapeutic range. This represents an example of the use of modern scientific methodology to verify traditional Mongolian medicine theory, and studies like this should facilitate the incorporation of Mongolian herbal medicines into clinical applications.

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