International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:6, No:12, 2012

Effect of *Geum Kokanicum* Total Extract on Induced Nociception and Inflammation in Male Mice

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***Abstract*—**The aim of this study is evaluating the antinociceptive and anti-inflamatory activity of *Geum kokanicum*. After determination total extract LD50, different doses of extract were chosen for intrapritoneal injections. In inflammation test, male NMRI mice were divided into 6 groups: control (normal saline), positive control (Dexamethasone 15mg/kg), and total extract (0.025, 0.05, 0.1, and 0.2 gr/kg). The inflammation was produced by xylene-induced edema. In order to evaluate the antinociceptive effect of total extract, formalin test was used. Mice were divided into 6 groups: control, positive control (morphine 10mg/kg), and 4 groups which received total extract. Then they received Formalin. The animals were observed for the reaction to pain. Data were analyzed using One-way ANOVA followed by Tukey-Kramer multiple comparison test. LD50 was 1 gr/kg. Data indicated that 0.5,0.1 and 0.2 gr/kg doses of total extract have particular antinociceptive and anti-inflammatory effects in a comparison with control ($P<0.001$). The most effective dose was 0.2 gr/kg which did not show any significant difference in a comparison with positive control. Results indicated that total extract can inhibit nociception in the first and second phase. The antinociceptive effects in high doses are the same as morphine as a strong analgesic substance. TLC chromatography indicated presence of steroids and triterpenoids in this plant. The effects of extract may be related to presence of these compounds.

***Keywords*—**Anti-inflammatory, Antinociceptive, *Geum kokanicum*, Mice.

I. Introduction

AS a result of adverse side effects caused by NSAIDs, tolerance and dependence induced by opiates the use of these drugs as anti-inflammatory and analgesic agents have not been successful in all cases. Therefore, new anti-inflammatory and analgesic drugs lacking those effects are being searched all over the world [1]. In this regard, nowadays there has been increasing interest in remedy of diseases by herbal medicines. But based on our wide search few studies have been done on chemical constituents and pharmaceutical effects of *G. kokanicum*.

Geum kokanicum Regel et Schmath, a member of the Rosaceae family is a perennial rhizomatous plant. This herb which grows in moist and high altitude regions is endemic of Iran. *G. kokanicum* has been traditionally used for treatment of diarrhea and other gastrointestinal disorders [2].

Faramarzi et al. indicated strong antimicrobial and antifungal effects of *G. kokanicum* essential oils [3]. Also results of another study showed that total extract and methanol fraction of dried rhizomes possess antimicrobial activity against *Staphylococcus aureus* and *Staphylococcus epidermitus* [4]. There is a report of potent anti-invasive characteristic of the polar *G. kokanicum* root extract which might be effective in anticancer treatments [5].

According to our knowledge, however other species of Geum such as *G. Japonicum, G. urbanum* have identified for their anti-inflammatory activity [6], [7] but nothing have been done to discover these properties in *G. kokanicum.* Therefore the aim of present study was assessing the anti-inflammatory and anti-nociceptive effects of *G. kokanicum* total extact.

II. Materials and Methods

A. Plant Material

Plant was collected from Rouin (North Khorasan Province of Iran) in June 2011 during flowering season. The sample was botanically identified by Department of Pharmacognosy, Tehran University of Medical Sciences, Iran. All parts of plant including aerial parts and roots were dried in shadow and ground to a fine mixture. Then 100 g of powdered plant was macerated exhaustively at room temperature with a hydroalcohol solution (ethanol/water 80% v/v) for 3 days and, subsequently the extract was concentrated by a rotary evaporator. For injection the extract were freshly dissolved in normal saline.

B. Animals

Male NMRI mice weighing 20-25 g were obtained from Razi institute (Karaj, Iran). The animals were housed in standard laboratory conditions and allowed access to water and food *ad libitum.* They were maintained under constant temperature and in a 12h light-dark cycle at environmental temperature of 21 ± 2°C. The experimental protocol was approved by the animal care review committee of TUMS (Tehran University of Medical Sciences).

C. Determination of LD50 (Lethal Dose)

Mice were accidentally divided into 10 separated groups of 10 animals each, and they received different doses of extract intraperitoneally. The number of deaths was counted at 48hr after treatment. LD50 value was calculated by Logit method

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[8]. Applied doses in experiments were base on 0.1 g/kg LD50 and its upper and lower doses.

D. Xylene-induced Ear Edema

The method described previously by Atta and Alkofahi [9] was used. Male mice were divided into groups of eight mice each. After 30 min of the i.p. injection of extract at doses of 0.025, 0.05, 0.1, and 0.2 g/kg body wt., 0.03 ml of xylene was applied on the anterior and posterior surfaces of the right ear. The left ear was considered as control. Control animals received (normal saline) or dexamethasone (15 mg/kg). Two hours after xylene application, the mice were sacrificed and both ears were removed. Circular sections of both treated and untreated ears were taken using a cork borer with a diameter of 7 mm and weighed. The difference in weight between left untreated ear sections and right treated ear section was calculated.

E. Formalin Test

The method described previously by Hunskaar and Hole [10] was used. Pain was induced by injecting 0.02 ml of 2.5% formalin (40% formaldehyde) in distilled water in the subplantar of the right hind paw. Male mice were divided into groups of eight mice each. Extract was administered intraperitoneally at doses of 0.025, 0.05, 0.1, and 0.2 g/kg body wt., 30 min before formalin injection. The control group received the same volume (normal saline). Morphine was used as positive control (10 mg/kg i.p.). The animals were observed to evaluate the licking time (an index of nociception) during the first phase, Neurogenic (0-5 min), and the second phase, inflammatory (15-30 min), after formalin injection.

F. Phytochemical Screening

Phytochemical screening of the extracts was performed with thin layer chromatography on TLC aluminum sheets (Merck $60F_{254}$). Chloroform/methanol (9:1) was used as solvent of the extract. The spots were visualized under UV light at 254 and 365 nm.

G. Statistical Analysis

Results were analyzed using One way ANOVA followed by Tukey- Kramer multiple comparison test. The data were expressed as mean values ± S.E.M. and difference between the means of treated and control groups was considered significant at $P<0.05$.

III. Results

Pereliminary phytochemical test by TLC indicated that total extract of *Geum kokanicum* contains steroid and triterpenoid compounds.

Topical application of different doses of extract (except 0.025 g/kg body wt.) to the mouse ear resulted to potent suppression ($P<0.001$) of acute edema induced by xylene (Table I). These responses were dose-dependent. The most effective dose in inhibition of inflammation was 0.2 g/kg.

In formalin test, all doses of extract (except 0.025 g/kg body wt.) showed significant effects in blockade of both first (0-5 min) and second phases (15-30 min) of response as compared with control. Therefore licking activity was inhibited in a dose-dependent manner. The most potent response produced by 0.2 g/kg of extract (Table II).

TABLE I
EFFECT OF *GEUM KOKANICUM* EXTRACT ON XYLENE-INDUCED EAR EDEMA IN MICE

Treatment (dose)	Ear swelling (mg)	Inhibition (%)
Control	2.8 ± 0.11	——
Dexamethasone (15 mg/kg)	0.92 ± 0.1 *	67.1
Extract (0.025 g/kg)	2.5 ± 0.14	10.7
Extract (0.05 g/kg)	1.95 ± 0.13*	30.3
Extract (0.1 g/kg)	1.55 ± 0.11*	44.6
Extract (0.2 g/kg)	1.28 ± 0.11*	54.3

Values are the mean ± S.E.M., *($P < 0.001$) compared to control. Differences between groups were statistically analyzed by One-way analysis of variance (ANOVA) followed by Tuky- Kramer multiple comparison test (n=8).

TABLE II
EFFECT OF *GEUM KOKANICUM* EXTRACT ON FORMALIN-INDUCED PAIN TEST IN MICE

	Licking Times (s)			
Treatment (dose)	0-5 min	Inhibitation (%)	15-30 min	Inhibitation (%)
Control	44.62 ± 2.7	——	17.62 ± 1.8	——
Morphine (10m g/kg)	7.62 ± 0.22*	82.9	2.75 ± 0.42*	84
Ex. (0.025 g/kg)	40 ± 2 67	10.3	14.75 ± 1.54	16.2
Ex. (0.05 g/kg)	30.7 ± 2.72*	31.8	9.87 ± 0.81*	44
Ex. (0.1 g/kg)	18.12 ± 1.15*	59.4	6.5 ± 0.68*	63.11
Ex. (0.2 g/kg)	10.37 ± 0.7*	76.65	3.62 ± 0.41*	79.4

Values are the mean ± S.E.M., *($P < 0.001$) compared to control. Differences between groups were statistically analyzed by One-way analysis of variance (ANOVA) followed by Tuky- Kramer multiple comparison test (n=8), Ex=extract

IV. Discussion

In this research for the first time the antinociceptive and anti-inflammatory effects of *G. kokanicum* total extract have evaluated by formalin test and xylene-induced ear edema.

The LD50 value of total extract was determined 1g/kg, therefore according to toxicity classification [8], it is slightly toxic.

Acute inflammation produced by topical application of the xylene to the mouse ear. Xylene causes instant irritation of ear, which leads to fluid accumulation and edema characteristic of the acute inflammatory response [9]. Suppression of this response probably indicates the acute anti-inflammatory effect of extract. In this research total extract revealed strong anti-inflammatory activity against acute inflammation. Also the total extract had remarkably showed antinociceptive activity in formalin test which was in a dose-dependent manner. Formalin test has two phases. The pain in the early phase is caused due to the direct stimulation of

sensory nerve fibers by formalin (acute or neurogenic pain) while the pain in the late phase is due to inflammatory mediators like histamine, PGs, serotonin and bradykinin [11]. The first phase is sensitive to opioids such as morphine which acts through central nervous system. But the second phase (chronic or inflammatory pain) is inhibited by both NSAIDs (non steroidal anti-inflammatory drugs) and opioids. The antinociceptive behavior of extract in two phases may be represents the central analgesic properties. However the existence of the peripheral analgesic activity is not clear yet and need to more experiments will be done. Action of peripheral anti-inflammatory drugs such as NSAIDs, is inhibition of inflammatory mediator's synthesis like prostaglandins as well as receptor blockade [12].

As preliminary phytochemical analysis indicated, the extract contained steroid and triterpenoid compounds which are documented anti-inflammatory and antinocicepyive constituent of some plants [13]-[15]. Also investigation of roots and rhizomes essential oils showed 17 different compounds, between them eugenol and myrtenol were the most active ingredients [4]. Some experiments on other plants like cinnamon, cloves, sage, and oregano which possess these phenolic compounds showed anti-inflammatory and antinociceptive activity [16]. It was reported that both oral [17] and intraperitoneal [18] administration of eugenol cause anti-inflammatory and peripheral antinociceptive activities in mice. The effect of eugenol might be related to inhibition of prostaglandin synthesis and release of other endogenous mediators like cyclooxygenase-2 (COX-2).

V. Conclusion

In conclusion, the results of the present study provide evidence for the antinociceptive and anti-inflammatory activity of the *Geum kokanicum* in acute inflammation and acute and chronic pain. Some constituents such as steroids, triterpenoids and essential oils contained in the plant especially eugenol may be responsible for these effects. However there is a need for further studies to understand the mechanisms of the action of *G. kokanicum* active ingredients.

Acknowledgment

The researcher would like to gratitude Ashtian Islamic Azad University for their supports.

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