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**Research Article****Investigation of the effects of the essence and extract of *Allium jesdianum* on the activity of COX-1 and COX-2 enzymes**

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**Running title: Anti-inflammatory effects of *Allium jesdianum***

**ABSTRACT**

Recent studies on the analgesic effects of *Allium jesdianum* herb across different types of pain including formalin test biphasic pain, have suggested the probability of effectiveness of the extract of this herb on cyclooxygenase enzymes (COX). In this study we investigated the effects of the essence and extract of *A. jesdianum* on COX-1 and COX-2 in human fresh blood. For this experimental study, *A. jesdianum* collected from Sefidkouh region in Khorramabad of Iran. Extract and essence (the leaves of the plant) were prepared at various concentrations 0.5-6 mg/ml and 0-100 mg/ml respectively. The percentage of activity of COX-1 and COX-2 enzyme was measured by percentage of production of TXB<sub>2</sub> and PGE<sub>2</sub>. Solvents Dimethyl sulfoxide (DMSO) and Phosphate-buffered saline (PBS) were used as control for comparing with extract or essence effects. Also indomethacin was used as a positive control. The extract (59±8.2; P<0.05) and essence (61±8.7; P<0.05) inhibited COX-1 activity. The extract and essence inhibited COX-2 activity, but it was not significant (P>0.05). Further, all doses of *A. jesdianum* essence inhibited the platelet aggregation (P<0.05). It can be stated that the essence and extract of *A. jesdianum* herb had inhibitory effects on COX-1 enzyme, where the extract was also able to inhibit platelet aggregation.

**Keywords:** Anti-inflammatory agents, Medicinal plants, Pain, Platelet aggregation.

**INTRODUCTION**

Currently, to control pain, non-steroidal anti-inflammatory drugs (NSAIDs) along with opioid analgesic drugs are used. These medications have numerous side effects. NSAIDs cause disturbance in gastrointestinal (GI) tract, kidney damage, and increased sensitivity reactions. Opioids bring about nausea, constipation, attenuation of respiration, and if chronically used, dependence and tolerance [1, 2].

Therefore, finding newer compounds with fewer side effects considering anti-inflammatory drugs has begun since 1960s [3]. As drugs with a natural origin generally bring about fewer side effects, it necessitates research on painkiller drugs with a plant-based origin [4, 5]. Medicinal plants as a rich source of natural compounds have been used in traditional medicine and studied for their effects [6-12]. Actually,

traditional medicine and herbs can be suitable sources for finding new drugs [13-17]. In our previous study, we found the analgesic and anti-inflammatory effect of *Allium jesdianum* on Rat [18]. This plant belongs to Alliaceae family [19] and is native to Iran. It grows at altitudes of 1800-2600 m, with its maximum height reaching 50 cm. It is traditionally used for problems including mitigating the pain of kidney stone and abdominal pain. Although these effects and the results of previous studies suggest the analgesic and anti-inflammatory properties of the extract of this herb [18], its mechanism of action is unknown. Further, as the anti-inflammatory effects of this herb have been comparable to those of sodium salicylate, a non-steroidal anti-inflammatory drug [18] which acts upon cyclooxygenase (COX) enzymes [20], it is possible that this herb has also a similar mechanism. The therapeutic effects and side effects of NSAIDs emerge in response to inhibition of COX enzymes. So far, two iso-enzymes have been discovered for this enzyme. COX-1 which exists in constitutive form and plays a role in physiological actions [21], where inhibition of this enzyme by NSAIDs causes decreased production of PGI<sub>2</sub> and PGE<sub>2</sub>, though it has the side effects of development of peptic ulcers and renal complications. On the other hand, COX-2 is in inducible form and plays a role in transmission of pain message and inflammation, where COX-2 inhibitors are healthier drugs and develop fewer side effects [1]. Therefore, finding drugs that have a more specific inhibitory effect on COX-1 is very valuable. Some NSAIDs including aspirin also cause inhibition of platelet aggregation and are useful in the treatment of cardiovascular diseases [22]. Thus, finding herbs that both inhibit COX and platelet aggregation enhances its advantage remarkably. Accordingly, we investigated the effects of the essence and extract of *A. jesdianum* on COX-1 and COX-2 isoforms, followed by evaluation of the effect of the extract on platelet aggregation.

#### **MATERIALS AND METHODS:**

In this experimental study, human blood was taken from healthy male volunteers who do not

have any inflammatory disease and at least one week have not received any NSAIDs. These individuals were referred to fellow sampling and the randomly selected number of samples for testing. In continue blood samples were taken and tested in treatment, negative and positive control groups. This study gained approval from ethics committee of Lorestan University of Medical Sciences in Jan, 2015. All volunteers filled out their informed written consent to participate in the study (LUMS.REC.1395.168).

#### **Preparation of the essence and extract:**

*A. jesdianum* was collected off an altitude of 2200 m from Sefidkouh region in Khoramabad in late April 2016. The plant was coded in the Herbarium of agriculture faculty at Lorestan University. The leaves of the plant were separated and then washed with cold water and then dried at room temperature and in shade. The flowers of the plant were separated from its leaves. A total of 100 g of dry plant was soaked in water-alcoholic solution (1:3) for extraction. The extraction was performed by Soxhlet device, after which solvent distillation was performed through rotary action. The prepared extract was kept in refrigerator until consumption. To prepare the essence, 40 g of leaves powdered by Clevenger apparatus through distillation with water, which was obtained as 2.20±0.05% (v/w).

#### **Preparation of blood sample:**

Human fresh blood was prepared using vacutainer free of natural anticoagulant from six volunteers who had no diseases and had not received any NSAIDs for at least two weeks (men 25-40 years old). The obtained sample was applied for investigation of COX-1 and COX-2 enzymes.

#### **Measurement of complete blood human COX-1:**

480 µl of blood along with 10 µl of the any one of treatments (extract of *A. jesdianum*, essence of *A. jesdianum*, and indomethacin)/control (in DMSO/saline) was poured into deep plates. The contents of the tube were exposed to 37°C in incubator for 1 h. Next, the samples (at 2250 g or 3700 rpm for 10 min at 4°C) were centrifuged in order for the serum to be obtained. The serum solution was mixed with methanol for

sedimentation of proteins so that the proteins will precipitate. The collected supernatant was dried by N<sub>2</sub> gas and the value of TXB<sub>2</sub> was measured by immunoassay method (R&D systems, Germany [23-25]).

#### Measurement of complete blood human COX-2:

The samples were diluted with lipopolysaccharide LPS in phosphate buffer (PBS) and then kept in incubator for 24 h. After that, with condition similar to measurement of COX-1, plasma was centrifuged and after precipitation of proteins, the dried supernatant was examined for collection of PGE<sub>2</sub> using immunoassay method [23-25].

#### Platelet aggregation:

First platelet count was performed on human fresh blood sample. Then, ADP was added to platelet-rich plasma (PRP) inside a cuvette with constant heat. In this state, the platelets begin to aggregate and the initial turbidity of the solution increases, where the results is registered and evaluated in the form of a curve using a photometer as elevation of optical density (OD) versus time. The obtained maximum dose was added to PRP for mixing the extract and after six minutes, ADP was added in the final concentration, whereby the platelet aggregation was examined. The developed response was calculated as the maximum response obtained in relation with the control group.

#### Data analysis:

The blood samples used for different experimental groups including extract, essence, indomethacin, DMSO and PBS groups. The number of samples was 6 in each group. The comparison of means was performed by unpaired t-test, one-way analysis of variance, and posttest Tukey statistical tests. If  $p < 0.05$ , then the data were considered to be significant.

## RESULTS:

Inhibition of COX activity in human complete blood has changed into a standard measurement instrument for selective inhibition of COX-1 and COX-2 activity. The degree of production of TXB<sub>2</sub> in complete blood is considered to be a suitable index for measurement of the activity of COX-1, while PGE<sub>2</sub> production rate is regarded

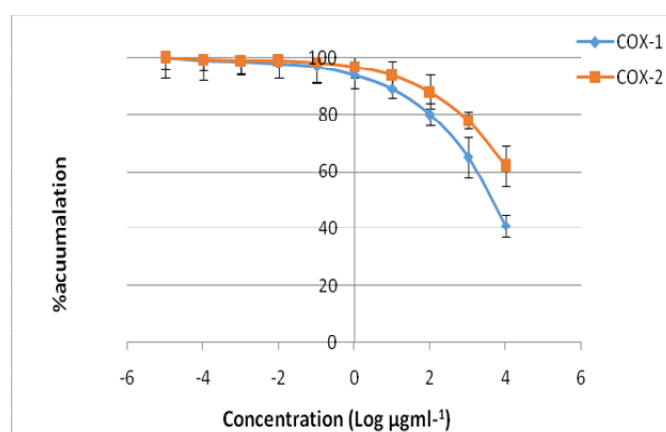
as a suitable index for activity of COX-2. Percentage of inhibition of cyclooxygenase enzymes over 50% was considered to be significant, whereas the cases below 50% were regarded to be of weak effect and insignificant.

The hydro alcoholic extract of *A. jesdianum* ( $59 \pm 8.2\%$ ) significant decreased the percentage of activity of COX-1 enzyme, when compared with DMSO/saline (Table 1). Further, it also reduced the degree of activity of COX-2 enzyme by ( $38 \pm 7.4\%$ ) in comparison with the control ( $P > 0.05$ ). The essence of *A. jesdianum* ( $61 \pm 8.7\%$ ) diminished the percentage of activity of COX-1 significantly ( $P < 0.05$ ), when compared with the control. It also reduced the percentage of activity of COX-2 enzyme by  $45 \pm 6.8\%$ , but it was not significant (Table 2).

**Table 1.** The percentage of inhibition of COX-1 and COX-2 enzymes in groups.

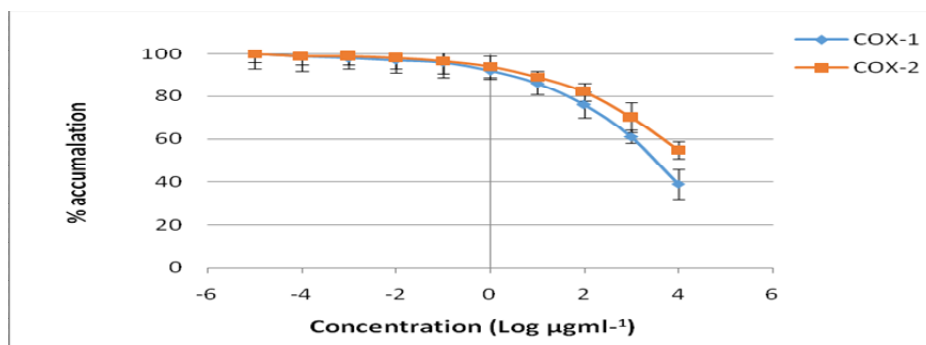
Treatment	% COX-1 inhibition (TXB <sub>2</sub> )	% COX-2 inhibition (PGE <sub>2</sub> )
Extract of <i>A. jesdianum</i>	$59 \pm 8.2^*$	$38 \pm 7.4$
Essence of <i>A. jesdianum</i>	$61 \pm 8.7^*$	$45 \pm 6.8$
Indomethacin	$80 \pm 1.9^{**}$	$69 \pm 2.4^{**}$
DMSO	-	-
PBS	-	-

The percentage of inhibition was expressed as Mean $\pm$ SD. The control groups for COX-1 and COX-2 were considered to be DMSO and PBS (as solvent), respectively. Indomethacin was used as positive control in the comparisons. The number of samples were 6 ( $n=6$ ). \* $P < 0.05$ , \*\* $P < 0.01$  in comparison with the control group.



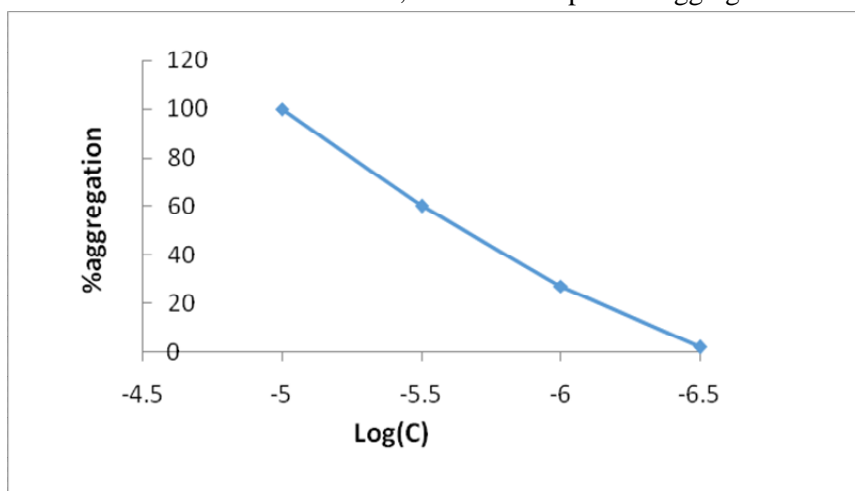
**Figure 1.** The effect of hydro alcoholic extract of *Allium jesdianum* on the activity of COX-1 and

COX-2 enzymes in complete blood (n=6). The control samples were compared with DMSO solution as

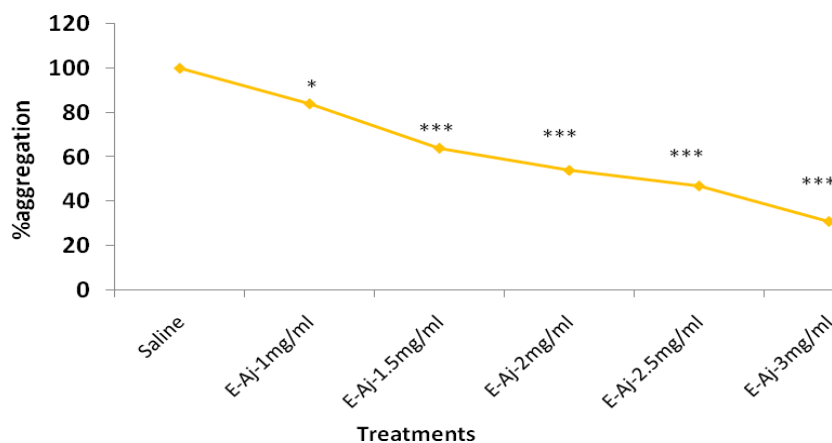


**Figure 2.** The effect of essence of *Allium jesdianum* on the activity of COX-1 and COX-2 enzymes in complete blood (n=6). The samples were compared with PBS solution as control.

Our results in Figure 3 indicate the increasing effect of different concentrations of ADP on platelet aggregation ( $EC_{50} = 2.43 \times 10^{-6}$  M). In contrast, Figure 4 shows the dose-dependent effect of different concentrations of *A. jesdianum* extract on platelet aggregation in response to ( $p < 0.01$ ) ADP ( $10^{-5}$  M), where with the increase in the dose of the extract, the extent of platelet aggregation has diminished.



**Figure 3.** The effect of ADP on induced platelet aggregation ADP. The points indicate Mean±SEM; the number of samples=6,  $EC_{50} = 2.43 \times 10^{-6}$ .



**Figure 4.** The inhibitory effect of *Allium jesdianum* extract on ADP. The points indicate Mean±SEM; E-Aj (Extract of *Allium jesdianum*), \* $p < 0.05$ , \*\*\* $p < 0.001$  in comparison with the control group.

## DISCUSSION

Prostaglandins are produced through the activity of COX-1 and COX-2 enzymes, which is a part of the production path of arachidonic acid. In this study, we showed that *A. jesdianum* act on COX activity and can be used for inhibition of prostaglandins. In the previous studies, *A. jesdianum* extract could produce antinociception in both phases of formalin tests and tail flick i.p. and i.t. administration to rat [18]. The first phase of test formalin and Tail flick test is high sensitive to centrally acting analgesic drugs [26]. Our results indicated that both the essence and extract of *A. jesdianum* are able to significantly inhibit COX-1 and to some extent inhibit COX-2, though these effects were not observed at minimum doses. In spite of the difference in the extent of inhibition of COX enzymes, both forms of the studied herb had an inhibitory effect, though this effect was not very strong, but was significant. In the previous study, we also investigated two types of pain, i.e. acute pain and chronic (inflammatory) pain with formalin test [18]. In this model the extract had caused diminished both phase of formalin test. Second phase of formalin test related to COX-2 enzyme activity and second phase of formalin was effected by extract of *A. jesdianum* (previous study). In any case, the analgesic effects in previous studies suggest the inhibitory effects of the extract of this herb on acute and inflammatory pains. Further, considering the findings of this study, the analgesic and anti-inflammatory effects of the extract can be attributed to the inhibitory effects of COX enzymes, though further supplementary studies seem to be required for certainty of these findings. In this study essence and extract of *A. jesdianum* inhibited COX-2 activity but not significant, but it significantly inhibited COX-1 activity. In our previous studies, we analyzed the active ingredients of the hydro alcoholic extract of this herb, where considering the results of this study, conductance of complementary studies on the constituent compounds and their interaction with COX enzymes help in discovery of a more accurate mechanism. On the other hand, the extract and essence of *A. jesdianum* caused

inhibition of platelet aggregation in a dose-dependent fashion. This effect is also observed in drugs such as aspirin which is a useful NSAID in cardiovascular diseases. Aspirin as a NSAIDs are able to inhibit platelet aggregation and are effective for COX enzymes [27].

## CONCLUSION

Considering the effects of the extract and essence on inhibition of COX enzymes and their effects on inhibition of platelet aggregation, the inhibitory mechanism of COX of *A. jesdianum* extract can be considered to be similar to that of NSAIDs, which is more powerful for COX-1 than for COX-2. Note however that the necessity of conductance of supplementary studies should not be neglected.

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