Astragalus fasciculifolius manna; antinociceptive, anti-inflammatory and antioxidant properties in mice

Mehrdad Shahrani, Najmeh Asgharzadeh, Akram Torki, Shirin Asgharian, Zahra Lorigooini*

Medicinal Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran

*Correspondence to
Zahra Lorigooini, Ph.D,
Email; gueini.z@skums.ac.ir

Abstract
Introduction: Inflammation is one of the body’s natural defence mechanisms alongside the cells or tissues damage due to various stimuli or infections. Pain is one of the greatest common symbols associated with different diseases. Therefore, researchers are trying to find new medications that bring patients relief with fewer side effects. Herbal medicine has been used for periods to treat acute and chronic pains.

Objectives: This research evaluated the anti-inflammatory, antinociceptive and antioxidant properties of Astragalus fasciculifolius manna extract.

Materials and Methods: The anti-inflammatory and antioxidant potency of the extract were determined by xylene test and ferric reducing antioxidant power assay.

Results: Astragalus fasciculifolius manna extract at doses of 400, 800 and 1200 mg/kg significantly reduced acetic acid-induced writhing and formalin-induced paw licking (late and early phase) but did not affect mechanical hyperalgesia caused by heat. Moreover, naloxone failed to inhibit the antinociceptive activity of the extract. The extract of A. fasciculifolius manna also exhibited high anti-inflammatory activity against xylene induced ear edema. Treatment of mice with the extract did not improve serum antioxidant capacity.

Conclusion: Results of the present research showed that A. fasciculifolius manna can prevent pain and inflammation because of the presence of high amount of saponin.

Introduction: Pain is one of the most common signs associated with different diseases and represents a major health and economic burden for society. It may lead to disability and reduction of the person's quality of life. The estimates of chronic pain prevalence range from 8 to over 60% depending on the population. There are several different types of drugs available to treat and alleviate pain (1). Present analgesic drugs, despite their confirmed pain-relieving effects, have significant side effects containing gastrointestinal complications, kidney damage, respiratory depression and emesis. They can also lead to physical and mental dependence (2). Researchers are therefore trying to find new medications that bring patients relief with fewer side effects. Herbal medicine has been used for centuries to treat acute and chronic pains (3).

Inflammation is one of the body’s natural defence mechanisms alongside cells or tissues damage either because of various stimuli (chemical, mechanical and thermal) or infections. Prostaglandins and pro-inflammatory cytokine play important roles as local messengers in the generation of the inflammatory response (4). Pain, redness, heat, swelling and edema are key symptoms of inflammation. It has been shown that many anti-inflammatory drugs such as a non-steroidal anti-inflammatory drug (NSAID) can alleviate inflammation and therefore relieves pain (5). Long-term use of these drugs can lead to gastrointestinal tract ulceration, renal impairment and bleeding. Therefore, it is necessary to find new analgesic and anti-inflammatory drugs which do not cause NSAIDs like side effects. Some herbal medicine such as white willow bark,
curcumin (turmeric), green tea, pycnogenol (maritime pine bark), Uncaria tomentosa (cat’s claw) and capsaicin (chili pepper) have been reported to alleviate inflammation and associated pain by suppressing inflammatory cytokine and prostaglandin synthesis (6).

Astragalus is a large genus of flowering plant, with more than 3000 species belonging to the family Fabaceae. The plants of this genus are broadly distributed throughout the temperate and arid region of the world (7). They have a long history of use as herbal medicine in traditional and folk medicines (8). Antioxidant, anti-inflammatory, analgesic, immunoregulatory and anti-tumour effects of the extract and phytochemical components of some species such as Astragalus membranaceus have been reported in previous pharmacological studies (8-10). A. fasciculifolius is a species in the Astragalus genus that grows wild in Iran. There is no previous study on the analgesic, anti-inflammatory and antioxidants effects of this plant.

Objectives
The key points of this research were; 1) to evaluate the analgesic activity of A. fasciculifolius manna extract against chemical (acetic acid and formalin) and thermal (hot plate) induced pain in mice; 2) to determine anti-inflammatory effects of A. fasciculifolius manna extract against xylene induced inflammation in mice; 3) to evaluate the properties of A. fasciculifolius manna extract on serum total antioxidant potency in mice.

Materials and Methods

Extraction of plant
Dried Manna of A. fasciculifolius manna was acquired from a local herbal market located in Kerman, Iran. Then, it was scientifically confirmed by an expert botanist (Dr. H.A. Shirmardi), a voucher specimen (no.: 721) was registered for it at the Medicinal Plants Research Center. Powdered leaves were extracted with ethanol (50%) by maceration method. Then, after 72 h, the mixture was filtered by using Whatman filter paper No.1 and concentrated under vacuum by rotary evaporator. The resulted aqueous extract was then evaporated under vacuum at 40°C (11).

Determination of total phenolic content
Total phenolic content was assayed agreeing with the method of Maisuthisakul et al (12). Briefly, 0.1 mL of diluted extract (60°C methanol) was mixed with 0.5 mL of Folin–Ciocalteu as a reagent and 0.4 mL of 7.5% sodium carbonate solution. The mixture was incubated for 30 minutes in the dark place at laboratory temperature, and then the absorbance was read at 765 nm against blank. The percent inhibition was determined against blank at 517 nm. The percent inhibition of DPPH free radicals was computed from (A<sub>control</sub>-A<sub>sample</sub>/A<sub>control</sub>) ×100, where A<sub>blank</sub> is the absorbance of the control reaction, and A<sub>sample</sub> is the absorbance of the solution in the presence of herbal extract. The concentration causing 50% inhibition of DPPH free radicals (IC<sub>50</sub>) was computed by the regression equation for the concentration of extract and percentage inhibition (12).

Determination of total saponin content of the extract
Total phenolic content was assayed agreeing with the method of Maisuthisakul et al (12). Briefly, 0.1 mL of diluted extract (60°C methanol) was mixed with 0.5 mL of Folin–Ciocalteu as a reagent and 0.4 mL of 7.5% sodium carbonate solution. The mixture was incubated for 30 minutes in the dark place at laboratory temperature, and then the absorbance was read at 765 nm against blank. The percent inhibition was determined against blank at 517 nm. The percent inhibition of DPPH free radicals was computed from (A<sub>control</sub>-A<sub>sample</sub>/A<sub>control</sub>) ×100, where A<sub>blank</sub> is the absorbance of the control reaction, and A<sub>sample</sub> is the absorbance of the solution in the presence of herbal extract. The concentration causing 50% inhibition of DPPH free radicals (IC<sub>50</sub>) was computed by the regression equation for the concentration of extract and percentage inhibition (12).

Determination of antioxidant capacity by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) method
The antioxidant potency of A. fasciculifolius manna extract was determined by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. Two milliliters of the extract was added to 2 mL of 0.1 mM DPPH- ethanol solution and mixed. After incubation for 15 minutes at laboratory temperature, the absorbance was determined against blank at 517 nm. The percent inhibition of DPPH free radicals was computed from (A<sub>control</sub>-A<sub>sample</sub>/A<sub>control</sub>) ×100, where A<sub>blank</sub> is the absorbance of the control reaction, and A<sub>sample</sub> is the absorbance of the solution in the presence of herbal extract. The concentration causing 50% inhibition of DPPH free radicals (IC<sub>50</sub>) was computed by the regression equation for the concentration of extract and percentage inhibition (12).

Experimental animals
Male mice, weighing 25-30 g were kept under standard laboratory circumstances (12 h light/12 h dark cycle at 21± 2°C) with free access to water and standard laboratory food (2).

Hot-plate test
This section of the investigation, 48 animals were accidentally divided into six groups. Each group included 8 mice. The groups included distilled water (1 mg/kg), plant extract (400, 800, 1200 mg/kg), morphine (0.5 mg/kg), and naloxone (0.5 mg/kg) + extract at the most effective dose. One day before the hot plate tests, animals were allowed to acclimate to the turn-off hot plate apparatus for
3-5 minutes. Fifteen minutes after the drug injection, hot-plate test was done. The mice were placed on the hot plate with constant temperature of 48°C. The time between the placing of the animal and the first response (paw-licking, lifting or shaking and animal jumping) was measured as latency to reach pain threshold. Animals were tested on a hot plate every 15 minutes for 1 hour. To prevention of tissue damage, the time of test was less than 60. In addition, the experiment was performed only once and the mice were removed from the study (2).

**Acetic acid test**
In this part, 48 mice were classified into 6 groups. Group 1 as the control group, received distilled water, groups 2, 3 and 4 received doses of 400, 800 and 1200 mg/kg of A. fasciculifolius manna extract, group 5 received morphine (1 mg/kg) as a standard analgesic drug and group 6 received naloxone (1 mg/kg as an opioid antagonist) + effective dose of the extract. Then, 30 minutes after intraperitoneal (IP) injection of the drugs, 100 mg/kg of %1 acetic acid was injected into each mouse to induce abdominal writhing. Then, the total number of abdominal writhing was recorded during 30 minutes (2).

**Formalin test**
Animals were divided into 6 groups like the acetic acid test groups. Fifteen minutes after drugs injection, 20 µL of formalin (2%) was administrated (into the right hind paw) and behavioural response to pain (lifting and licking of the same paw) was checked and recorded for 30 minutes (every 5 minutes). The first 5 minutes was considered as the acute phase and the last 5 minutes was considered as the chronic phase (2).

**Xylene test**
Anti-inflammatory effect of the extract was investigated by the xylene test based on the method of Torres-Rêgo et al (14). 6 groups of 8 individuals were treated as follows; group 1 received intraperitoneal injections of saline, group 2 received dexamethasone (13 mg/kg, IP), groups 3, 4 and 5 received doses of 0.5, 1 and 2 mg/kg of extract by an intraperitoneal injection. Inflammation was created by intradermal injection of xylene (0.03 mL) into the frontal and dorsal sides of the right ear. All the drugs were injected 15 min before the xylene injection. After 2 hours, mice were sacrificed and their ears were cut. Circular pieces (7 mm) of both the left (untreated) and right (treated) ears were prepared and weighed. Inflammation was determined as the difference of weight between the two earpieces.

**Serum antioxidant capacity**
After behavioural test, by cardiac puncture, blood samples were taken from each animal, and the serum was separated by centrifugation. The ferric reducing ability of plasma (FRAP) assay was applied to measure the total antioxidant capacity of the serum. This technique is based on the ability of the serum to reduce ferric-tripyridyltriazine (Fe²⁺-TPTZ) to a ferrous form (Fe⁺⁺) which gives a complex (Fe⁺⁺- TPTZ) with blue colour and a maximum absorption at 593 nm (15).

**Results**

**Antioxidant properties of the plant**
Table 1 displays the antioxidant capacity of extract of A. fasciculifolius manna in DPPH method. The IC₅₀ value of this extract was found to be 223.57 µg/mL.

**Phytochemical contents of the extract**
The content of phenolic compounds in the A. fasciculifolius manna was 36.35 ± mg GAE/g extract while the contents of flavonoids and flavonols were negligible. The saponin content of the dried plant (25 g) was approximately 32% w/w.

**Hot-plate test**
The latency time of pain reactions to thermal stimuli in the hot plate test is displayed in Figure 1. Morphine as a standard analgesic drug significantly increased the response latency to pain at both 15 and 60 min of the test period (P<0.05). Doses of 400, 800 and 1200 mg/kg of the extract of A. fasciculifolius manna did not prolong the latency to reach pain threshold. At 0 and 60 minutes, there was a significant decrease in response latency to pain in extract treated groups (P<0.05).

**Formalin test**
Figure 2 shows the influence of A. fasciculifolius manna extract on formalin-induced nociceptive behaviour during phase I and phase II. In the first phase and final phase, the number of paw licking/lifting behaviours in the group of control was significantly higher than those in the group of morphine-treated (P<0.05). A. fasciculifolius manna extract significantly decreased both first and final phase formalin-induced pain behaviour (P<0.05). During the final phase, A. fasciculifolius manna extract reduces the paw licking/lifting behaviours in a dose-dependent mode. Moreover, the IP injection of naloxone (1 mg/kg) inhibited the analgesia induced by A. fasciculifolius manna extract only during the late phase.

**Acetic acid test**
Table 1. The antioxidant capacity of A. fasciculifolius extract in DPPH method

| Sample          | Concentration (µg/mL) | DPPH radical scavenging activity (%) | IC₅₀ (µg/mL) |
|-----------------|-----------------------|--------------------------------------|-------------|
|                 |                       |                                      |             |
| A. fasciculifolius | 200                   | 51.24                                | 223.57      |
|                 | 250                   | 52.96                                |             |
|                 | 300                   | 59.90                                |             |
|                 | 350                   | 67.91                                |             |

---

**Table 1. The antioxidant capacity of A. fasciculifolius extract in DPPH method**
Figure 3 displays the influences of *A. fasciculifolius* manna extract on abdominal writhing induced by acetic acid in mice. Morphine as the reference drug (1 mg/kg), significantly reduced the number of writhing produced by acetic acid (*P* < 0.05). The number of abdominal writhing was significantly lower in *A. fasciculifolius* manna extract treated groups compared with the acetic acid group (*P* < 0.05). There was not any statistically significant change between groups received different doses of the extract (*P* > 0.05). Doses of 400, 800 and 1200 mg/kg of *A. fasciculifolius* manna extract affected a time-dependent decrease in the number of writhing induced by acid acetic. As shown in Figure 3, pretreatment with naloxone (1 mg/kg, IP) did not inhibit the analgesia induced by *A. fasciculifolius* manna extract (*P* > 0.05).

**Xylene test**

Figure 4 shows the influence of *A. fasciculifolius* manna extract on the ear inflammation created by xylene in mice. Mouse ear edema was created by intradermal injection of xylene into the right ear. Dexamethasone (13 mg/kg, IP) significantly decreased the xylene induced ear edema in mice (*P* < 0.05). Intraperitoneal injection of the doses of 800 and 1200 mg/kg of *A. fasciculifolius* manna extract caused significant reductions in ear edema induced by xylene. Moreover, 1200 mg/kg of the extract displayed better activity of anti-inflammatory (*P* < 0.05).

**Serum antioxidant capacity**

Figure 5 shows the total serum antioxidant capacity of different groups. No significant difference observed between different experimental groups.

**Ethical issues**

All experimental and animal care procedures were performed in based on the guide for the care and use of laboratory animals and were accepted by research and ethics committee of Shahrkord University of Medical Sciences (ethical code; IR.SKUMS.REC.1394.74).

**Statistical analysis**

All information was shown as the mean ± SEM. One-way ANOVA followed by Duncan Multiple range test was
used to compare the means between experimental groups. Additionally, $P < 0.05$ was considered statically significant.

**Discussion**

We used tests of hot plate, acetic acid and formalin to study the antinociceptive activities of *A. fasciculifolius* manna extract. The anti-inflammatory and antioxidant actions of the extract were also determined by xylene test and FRAP.

The test of hot plate is suitable for estimation of the centrally acting analgesics which are recognized to increase the pain threshold of animal towards heat (16). This test is seemed to involve opioids and measures the compound reaction to an acute non-inflammatory nociceptive input (17). In this study, dose of 0.5 mg/kg of morphine considerably prolonged the reaction latency to thermally induced pain. Morphine is a centrally acting analgesic that binds to μ opioid receptors and therefore relieves pain (18). This study showed that *A. fasciculifolius* manna extract did not prolong the reaction time of mice to noxious thermal pain. This reveals that the extract of *A. fasciculifolius* is not working through the central nervous system.

The acetic acid-induced writhing test is considered as an animal model of inflammatory pain. It is utilized as a screening method for assessing analgesic or anti-inflammatory effects of natural agents (18). It has been recommended that acetic acid works by discharging endogenous inflammatory mediators or irritation of the visceral surface, which causes to the release of inflammatory mediators including amines (e.g., histamine, serotonin), kinins (e.g., bradykinin), prostanoids (e.g., prostaglandins) and cytokines (tumor necrosis factor alpha, interleukin-1β and interleukin-8) (19,20). Results of this study showed that the hydro-alcoholic extract of *A. fasciculifolius* significantly reduces the number of acetic acid induced abdominal constriction or writhing in mice. In addition, its painkilling activity is not inhibited by naloxone which indicates that the opioid receptors are not involved in the analgesic effects of the extracts. The antinociceptive influence of the extract against acetic acid-induced pain can because of either its action on visceral receptors sensitive to acetic acid or inhibition of the prostaglandin and pro-inflammatory cytokine synthesis in the gastrointestinal tract (21).

In our study, we used xylene test to assess the anti-inflammatory influence of *A. fasciculifolius* manna extract. Xylene induces the discharge of inflammatory mediators from sensory neurons. These mediators, in turn, work on peripheral target cells, for example mast cells and immune cells to produce inflammation characterized by warmth, redness and edema (22,23). In our study, *A. fasciculifolius* manna extract played a significant anti-inflammatory effect against xylene induced ear edema. Thus, the anti-inflammatory effect observed for *A. fasciculifolius* manna extract can be related to the inhibition of inflammatory mediators. Several medicinal plants were also reported to be effective in inhibiting xylene induced ear edema (24,25).

We also used test of formalin which is a valid and reliable test of nociception and inflammatory pain (2,4) to assess the analgesic influence of *A. fasciculifolius* manna extract. The first phase of formalin-induced nociception is because of the direct chemical stimulation of the nociceptors and the late phase is due to the inflammation of peripheral tissue. It was told that substance P and bradykinin contribute in the first phase, whereas histamine, serotonin, prostaglandins, nitric oxide and bradykinin are participated in the final phase. The results of our study exhibited that *A. fasciculifolius* hydro-alcoholic extract produces considerable analgesic activity in both first and final phase of the test of formalin. It can be concluded that the extract works by inhibiting the discharge and/or action of these substances because the extract showed significant inhibitory effects in both phases.

The phytochemical screening of *A. fasciculifolius* manna extract shown the presence of high amount of saponins. Many saponins isolated from medicinal plants have been informed to have antinociceptive and anti-inflammatory activities. For example, saponins separated from roots of *Chlorophytum borivilianum* significantly reduced histamine- and carrageenan-induced inflammation in rats (26). Saponin from *Quillaja saponaria* was also found to reduce the carrageenan induced inflammation in mice (27). The saponin fraction of *Zizyphus lotus* was also reported to alleviate paw edema and pain by inhibition of the nitrite production (28). The anti-inflammatory properties of the saponins from *Entada phaseoloides* is attributed to the inhibition of the creation of nitric oxide and pro-inflammatory cytokines for example tumor necrosis factor alpha, interleukin-1β, interleukin-6 and interleukin-8 (29). Wang et al also found that pirostane-steroidal saponins from *Bletilla striata* show significant anti-inflammatory effects against cyclooxygenase-1 and cyclooxygenase-2 (30). The antinociceptive effect observed for *A. fasciculifolius* manna extract in our study
can be attributed to the presence of higher amounts of saponin which possess anti-inflammatory effects.

**Conclusion**

In conclusion, this study exhibited that *A. fasciculifolius* manna extract attenuates acid acetic- and formalin-induced pain in mice. It also inhibits the xylene evoked inflammation.

**Authors’ contribution**

MSH and ZL designed the research. NA conducted experimental part of animal study. Sha and AT analyzed data and prepared the final draft of the manuscript. All authors read and signed the final manuscript.

**Conflicts of interest**

The authors declare that they have no competing interest.

**Ethical considerations**

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission and redundancy) have been completely observed by the authors.

**Funding/Support**

This work was supported by a grant (#1874) from Shahrekord University of Medical Sciences.

**References**

1. Phillips CJ. The Cost and Burden of Chronic Pain. *Rev Pain*. 2009;3:2-5. doi: 10.1177/2049463709300102

2. Singal A, Anjaneyulu M, Chopra K. Modulatory role of green tea extract on antinociceptive effect of morphine in diabetic mice. *J Med Food*. 2005;8:386-91. doi: 10.1089/jmf.2005.8.386

3. Scholz J, Woolf C. Can we conquer pain? *Nat Neurosci*. 2002;5:1062-7. doi: 10.1038/nn942

4. Conforti F, Sosa S, Marrelli M, Menichini F, Statti G, Uzunov D. The protective ability of Mediterranean dietary plants against the oxidative damage: the role of radical oxygen species in inflammation and the polyphenol, flavonoid and sterol contents. *Food Chem*. 2009;112:587-94. doi: 10.1016/j. foodchem.2008.06.013

5. Aguilar J, Rojas P, Marcelo A, Plaza A, Bauer R, Reininger E. Anti-inflammatory activity of two different extracts of *Uncaria tomentosa* (Rubiaceae). *J Ethnopharmacol*. 2002;81:271-6. doi: 10.1016/s0378-7417(02)00093-4

6. Maroon J, Bost JW, Maroon A. Natural anti-inflammatory agents for pain relief. *Surg Neurol Int*. 2010;1:23-35. doi: 10.4103/2152-7806.73804

7. Avuduk S, Mitaine-Offer AC, Alankus-Caliskan O, Miyamoto T, Senol S, Lacaille-Dubois M. Triterpenoid glycosides from the roots of *Astragalus flavescens*. *J Nat Prod*. 2008;71:141-5. doi: 10.1021/np0703500

8. Jiang JB, Qiu JD, Yang LH, He JP, Smith GW, Li AQ. Therapeutic effects of astragalus polysaccharides on inflammation and synovial apoptosis in rats with adjuvant-induced arthritis. *Int J Rheum Dis*. 2010;3:396-405. doi: 10.1111/j.1756-185X.2010.01355.x

9. Ma Z, Yang Z. Scavenging effects of Astragalus and *Gynostemma pentaphyllum* with its product on O2- and .OH. *Zhong Yao Cai*. 1999;22:303-6.

10. Yang QM, Lu LT, Zhou AW, Wang B, He G, Chen MZ. Antinociceptive effect of astragalesides and its mechanism of action. *Acta Pharmacologica Sinica*. 2001;22: 809-12.

11. Pistelli L, Bertoli A, Lepori E, Morelli I, Panizzi L. Antimicrobial and antifungal activity of crude extracts and isolated saponins from Astragalus venicosus. *Fitoterapia*. 2002;73:336-9. doi: 10.1016/s0367-3260(02)00087-4

12. Maisuthisakul P, Suttajit M, Pongsawatmanit R. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. *Food Chem*. 2007;100:1409-18. doi: 10.1016/j.foodchem.2005.11.032

13. Massiot G, Lavaud C, Guillaume D, Men-Oliver LL. Reinvestigation of the Sapogenins and Prosapogenins from Alfalfa (*Medicago sativa*). *J Agric Food Chem*. 1988;36:902-9. doi: 10.1021/jf00083a005

14. Torres-Rêgo M, Furtado AA, Bitencourt MA, de Souza Lima MC, de Andrada RO, de Azevedo EP, et al. Anti-inflammatory activity of aqueous extract and bioactive compounds identified from the fruits of *Hancornia speciosa* Gomes (*Apocynaceae*). *BMC Complement Altern Med*. 2016;16:275. doi: 10.1186/s12906-016-1259-x

15. Bodera P, Stankiewicz W, Zawada K, Antkowiak B, Paluch M, Kieliszek J, et al. Changes in antioxidant capacity of blood due to mutual action of electromagnetic field (1800 MHz) and opioid drug (tramadol) in animal model of persistent inflammatory state. *Pharmacol Rep*. 2013;65:421-8. doi: 10.1016/j.pharep.2013.10.017

16. Hiruma-Lima C, Gracioso J, Bighetti E, Germanson Robinoue L, SouzaBrito A. The juice of fresh leaves of *Boerhavias distifusa* (Nygatinaeae) markedly reduces pain in mice. *J Ethnopharmacol*. 2000;71:267-74. doi: 10.1016/s0378-8741(99)00178-1

17. Zakaria Z, Ghanzi N, Nor R, Gopalan H, Sulaiman M, Jais A, et al. Antinociceptive, anti-inflammatory, and antiangiogenic properties of an aqueous extract of *Dicranopoteris linearis* leaves in experimental animal models. *J Nat Med*. 2008;62:179-87. doi: 10.1007/s11418-007-0224-x

18. Xu Q, Wang Y, Gao S, Shen Z, Wang Y, Yang L. Anti-inflammatory and analgesic activity of aqueous extract of *Flos populi*. *J Ethnopharmacol*. 2014;152:540-5. doi: 10.1016/j. jep.2014.01.037

19. Feng Y, Cui M, Willis W. Gabapentin markedly reduces acetic acid induced visceral nociception. *Anesthesiology*. 2003;98:729-33. doi: 10.1097/00000542-200303000-00023

20. Ribeiro R, Vale M, Thomazzi S, Paschoalato A, Poole S, Ferreira S, Cunha F. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. *Eur J Pharmacol*. 2000;387:111-8. doi: 10.1016/s0014-2999(99)00790-6

21. Anaya-Eugenio GD, Rivero-Cruz I, Bye R, Linares E, Mata R. Antinociceptive activity of the essential oil from *Artemisia ludoviciana*. *J Ethnopharmacol*. 2016;179:403-11. doi: 10.1016/j.jep.2016.01.008

22. Parveen Z, Deng Y, Saeed M, Dai R, Ahamad W, Wu Y. Antiinflammatory and analgesic activities of Theseus chinese Turcz extracts and its major flavonoids, kaempferol and kaempferol-3-O-glucoside. *Pharm Soc Jpn*. 2007;127:1275-9. doi: 10.1248/yakushi.127.1275

23. Zhang Z, Luo P, Li J, Yi T, Wang J, An J, et al. Comparison of the anti-inflammatory activities of three medicinal plants known as “Meidulouumi” in Tibetan folk medicine. *Yakugaku Zasshi*. 2008;128:805-10. doi: 10.1248/yakushi.128.805

24. Azab A, Nassar A, Azab AN. Anti-inflammation activity of natural products. *Molecules*. 2016;21(10):1321. doi: 10.3390/ molecules21101321

25. Rahman SM, Atikullah M, Islam MN, Mohaimenul M, Ahammad F, Islam MS, Saha B, Rahman MH. Anti-inflammatory, antinociceptive and antioxidant activities of methanol and ethyl acetate extract of *Hemigraphis alternata* leaves in mice. *Clinical Phytoscience*. 2019;5(1):16. doi: 10.1186/s40816-019-0110-6
26. Lande AA, Ambavade SD, Swami US, Adkar PP, Ambavade PD, Waghamare AB. Saponins isolated from roots of *Chlorophytum borivilianum* reduce acute and chronic inflammation and histone deacetylase. J Integr Med. 2015;13:25-33. doi:10.1016/s2095-4964(15)60157-1

27. Sarkhel S. Evaluation of the anti-inflammatory activities of *Quillaja saponaria* Mol. saponin extract in mice. Toxicology Reports. 2016;3:1-3. doi: 10.1016/S2095-4964(15)60157-1

28. Borgi W, Recio MC, Ríos JL, Chouchane N. Anti-inflammatory and analgesic activities of flavonoid and saponin fractions from *Zizyphus lotus* (L.) Lam. South African Journal of Botany. 2008;74:320-4. doi: 10.1016/j.sajb.2008.01.009

29. Xiong H, Zheng Y, Yang G, Wang H, Mei Z. Triterpene saponins with anti-inflammatory activity from the stems of *Entada phaseoloides*. Fitoterapia. 2015;103:33-45. doi: 10.1016/j.fitote.2015.03.001

30. Wang W, Meng H. Cytotoxic, anti-inflammatory and hemostatic spirostane-steroidal saponins from the ethanol extract of the roots of *Bletilla striata*. Fitoterapia. 2015;101:12-8. doi: 10.1016/j.fitote.2014.11.005