Original Article

Antinociceptive effect of palm date spathe hydroalcoholic extract on acute and chronic pain in mice as compared with analgesic effect of morphine and diclofenac

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Abstract

Backgrounds: In Persian traditional medicine, palm date spathe (PDS) is introduced as an analgesic. Therefore, this study was designed to investigate the analgesic effect of hydroalcoholic extract of PDS on acute and chronic pain in mice in comparison with diclofenac and morphine.

Materials and Methods: In this study, which was conducted in summer 2014, 220 male mice (20–30 g) were randomly divided into two categories, each consists of 11 groups as follows: A normal control group, a solvent (Tween 80) control group, 3 morphine positive control groups (2, 4 and 8 mg/kg), 3 diclofenac positive control groups (10, 20 and 30 mg/kg), and 3 main experimental PDS groups (2, 20, and 200 mg/kg). Hot plate was applied on animals in one category and writing test on the other category to assess acute and chronic pain, respectively.

Results: In the writing test, the average writing time and number of animals receiving a maximum dosage of morphine, diclofenac, and PDS were significantly less than the control group. In the hot plate test, only groups receiving different doses of morphine at different time points and those received 30 mg/kg diclofenac at 15 min after the intervention showed significant difference with the control group.

Conclusion: 200 mg/kg extract of PDS, revealed a significant analgesic effect on chronic pain, but it did not show any analgesic effect on acute pain.

Key Words: Mic, pain, palm date, phoenix dactylifera, spathe

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INTRODUCTION

Pain is a sign of diseases and acts as a warning mechanism to alarm a possible tissue injury.[1]

Nowadays, opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) are widely and increasingly used to relieve pain throughout the world.[2]

The use of analgesic drugs for pain relief revealed their side effects. For example, opioids can cause nausea, respiratory failure, consternation, and constipation, and if applied for a long time, it will be addictive. NSAIDs also can lead to gastrointestinal disorders, renal damage, and so on.[2,3] Due to the side effects of these drugs and the economical issues, it is important to conduct the research projects on finding new potential analgesic drugs with fewer side effects that can replace the present drugs.[4]

Due to progressive public interest in the use of medicinal plants, many researchers have been focused on the analgesic effects of various plants, which are mentioned as painkillers in traditional medicine.[5] Based on Persian traditional medicine, palm date spathe (PDS) distil, which is obtained from the inflorescence sheath of phoenix dactylifera, and is known as "taroone," is widely used as a healer remedy for lowering blood lipids, increasing breast milk, relieving tooth and joint pains, treating rheumatic diseases, strengthening the sexual power, and curing diarrhea and cramp, as well as sedative and hypnotic effects.[6] Chemically, PDS extract contains proteins, fats, regenerative and nonregenerative sugars, lignin, phenolic compounds, flavonoids, furfural, calcium pectate, 1,2-dimethoxy 4-methylbenzene, 3,4-dimethoxytoluene, camphor and Coumarin derivatives, phytosterols, amino acids, vitamins, moisture, and wood ash.[7-10] The anti-inflammatory and analgesic effect for some of these constituents has been evaluated.[11-13]

Based on the evidences in traditional medicine,[4] the results of a preliminary experimental trial study that confirmed the analgesic effect of PDS extract in a formalin-induced pain model in male rats,[14] and evidences for analgesic and anti-inflammatory effects of some spath's components, the present study, was conducted to evaluate the analgesic efficacy of PDS hydroalcoholic extract and compare its potency with the analgesic effects of morphine and diclofenac, in both chronic (using the writhing test) and acute (using the hot plate test) pain models in male mice.

MATERIALS AND METHODS

Animals

In this experimental trial study, which was conducted in summer 2014, 220 male albino mice (20–30 g), were selected from laboratory animal house in the Shahid Sadoughi University of Medical Sciences, Yazd, Iran, by simple sampling. Animals were kept in same environmental condition and randomly divided into two equal categories. Each category was divided randomly and equally into 11 groups under the following treatments: The distilled water control group and the solvent control group was received PDS extract solvent (20% solution of Tween 80), 3 test groups receiving 2, 20, and 200 mg/kg hydroalcoholic PDS extract, 3 positive control groups, were under the effect of 2, 4, and 8 mg/kg morphine sulfate, and the last 3 positive control groups were treated by 10, 20, and 30 mg/kg sodium diclofenac. Hot plate was applied on animals in one category and writing test on the other category to assess acute and chronic pain, respectively. In this study, animal handling was approved by the Institutional Ethical Committee and all efforts were made to minimize the animal's suffering in the experimental procedures.

Preparation of palm date spathe extract

In order to prepare the PDS extract, the fresh sheaths of palm inflorescence, taken from a Bam Mazafati palm tree (Phoenix dactylifera) in South Kerman Province (Iran), was dried at room temperature. Then, the dried pieces of spathe were pulverized by the electric mill. Two hundred gram of spathe powder was soaked in 1000 ml of 80° ethanol and kept in a black glass jar in a dark place at room temperature and was shaken at regular intervals. After 48 h, the mixture was extracted by a piece of clean cloth and passed through a paper filter. The yielded solution was then centrifuged at 4000 round for 10 min. The supernatant, as the crud spathe extract was kept in a flat glass vessel for 10 days until completely dried. Plant materials were approved by botanists in Bam Agriculture Research Centre, and a sample of crude extract was harvested in Yazd Herbal Medicine Research Centre. In order to prepare the solution for injection, the required amount of the PDS extract was weighed, and dissolved in 20% solution of Tween 80, and was kept in a refrigerator at a temperature of 4°C until the time of injection.[15]

Hot plate test

To assess the acute/tonic pain, a hot plate analgesia meter (Borjsanat co., Iran) was used. The device consists of an electrically heated surface which its temperature is controlled for 54–56°C. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a built-in stopwatch. The response latency is recorded before (baseline latency) and after 15, 30, 45, and 60 min following intraperitoneal administration of the standard drugs or PDS extract (test latency). In order to reduce the mice stress of being in the test device, before turning the hot plate apparatus on, each mice was

put in the device for 3 times with 5 min intervals. In the present study, the hot plate temperature was tuned on 54 ± 0.1°C and to prevent the tissue injury, 30 s cut-off was considered for this test.[16,17]

In order to eliminate the effect of individual variations, for each animal, the percentage of maximum possible effect (%MPE) in 4 times points (15, 30, 45, and 60 min after intervention) was calculated by following formula:

%MPE = (test latency – baseline latency)/ (cut-off-baseline latency) ×100

Writhing test

To assess the chronic pain in animals, 15 min after the treatment, the writhing test was carried out on each animal, including an intraperitoneal injection of 10 ml/kg of 0.7% acetic acid solution, and recording the number and duration of its writhes as a sign of chemical induced pain for 30 min.[18]

Statistical analysis

Data obtained in this study were considered as mean ± standard error of mean, analyzed by stat graph software (GraphPad Software, Inc., USA), using the two-way ANOVA, followed by Bonferroni *post hoc* test for comparing %MPE in different groups at 4 different time points and one-way ANOVA followed by Tukey's posttest to compare the number and the time course of writings in different groups. In this statistical analysis, $P < 0.05$ was considered as the level of significance.

RESULTS

Hot plate test

The average %MPE in response to heat stimulus in different groups, in the hot plate test, at intervals of 15 min is shown in Table 1.

Statistical analysis of %MPE in different groups at different time points showed that the animals receiving 8 mg/kg morphine at all times, and those received 30 mg/kg diclofenac, 15 min after the intervention, showed significant differences with control group. There was no significant difference between DW and TW groups in any time point. Also, animals receiving different doses of PDS extract did not show any significant difference as compared with DW and TW groups [Table 1].

Writhing test

In writhing test, the maximum average time of pain expression (in seconds), during a 30 min test interval, was observed in control group (212.5 ± 23.48) and the minimum average was for group receiving 200 mg/kg PDS extract (0.38 ± 0.16) [Figure 1]. At the same time, the maximum average of writhes numbers, was shown by group who received 10 mg/kg diclofenac (18.58 ± 2.09) and its minimum was for the group that received 200 mg/kg PDS extract (0.29 ± 0.13) [Figure 2]. In positive control and test groups animal's responses to painful stimulus, changed over time, and were dependent on the dosage of treatments. In an overall view, morphine, diclofenac, and PDS extract attenuate the pain induced by intraperitoneal injection of acetic acid, and the average time and number of writhes in animals who received the maximum doses of their medications were significantly lower than the control group ($P < 0.001$).

Regarding the time of writhing, there was no significant difference between the group that received 200 mg/kg PDS extract and those received 30 mg/kg

Table 1: Mean±SEM of animal's %MPE in different groups in the hot plate test (*n*=8)

Time sections	Groups										
	DW	TW	Mor2	Mor4	Mor8	Dic 10	Dic20	Dic30	PDS2	PDS20	PDS 200
15 min											
Mean	−0.89^{c,d}	4^{d}	10^{d}	27^{a,b}	16^{a}	2.1^{d}	6.5^{d}	16^{a}	5.2^{d}	2.3^{d}	1.8^{d}
SEM	2.7	2.2	2.6	4.4	4.3	2.6	1.7	6.4	3.2	1.5	1
30 min											
Mean	6.4^{c,d}	4.4^{c,d}	12^{d}	26^{a,b}	32^{a}	6.6^{d}	12^{d}	10^{d}	4.7^{c,d}	4.2^{c,d}	5.9^{c,d}
SEM	3.5	2.1	3.8	5.4	8.8	2.3	2	2.7	3.6	3	2.2
45 min											
Mean	4.2^{c,d}	4.8^{c,d}	14^{d}	26^{a,b}	32^{a}	6.5^{c,d}	9.6^{c,d}	13^{d}	7.4^{d}	4.5^{d}	6.4^{d}
SEM	1.8	1.4	1.9	2.7	3.6	1.8	1.5	1.8	2	1.6	1.7
60 min											
Mean	7.4^{d}	4.17^{d}	14.83	18.23	33.6^{a,b}	7.08^{d}	9.07^{d}	13.13	10.34^{d}	7.15^{d}	12.14^{d}
SEM	4.44	3.28	4.77	5.11	5.36	5.10	4.14	2.41	5.11	4.77	4.58

According to the two-way ANOVA followed by Bonferroni *post hoc* test, a, b, c, and d indicate significant differences ($P<0.001$) as compared with groups receiving DW, TW, Mor4, and Mor8, respectively. %MPE: Percentage of maximum possible effect, DW: Distilled water, TW: 20% Twin 80, Mor2: Morphine sulfate 2 mg/kg, Mor4: Morphine sulfate 4 mg/kg, Mor8: Morphine sulfate 8 mg/kg, Dic10: Diclofenac 10 mg/kg, Dic200: Diclofenac 20 mg/kg, Dic30: Diclofenac 30 mg/kg, PDS2: Palm date spathe 2 mg/kg, PDS20: Palm date spathe 20 mg/kg, PDS200: Palm date spathe 200 mg/kg, SEM: Standard error of mean

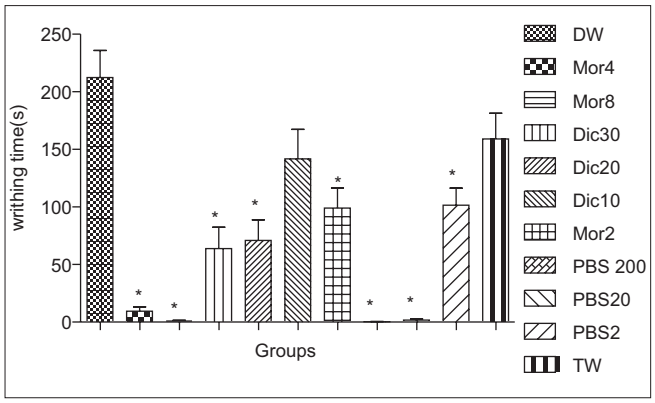

Figure 1: The effects of palm date spathe extract and positive control drugs on writhing time (second) as compared with control groups (n = 8). Based on the one-way ANOVA followed by Tukey's *post hoc* test, *inducatethe significant differences, as compared with the control groups (P < 0.01). DW: Distilled water, TW: 20% Twin 80, Mor2: Morphine sulfate 2 mg/kg, Mor4: Morphine sulfate 4 mg/kg, Mor8: Morphine sulfate 8 mg/kg, Dic10: Diclofenac 10 mg/kg, Dic200: Diclofenac 20 mg/kg, Dic30: Diclofenac 30 mg/kg, PDS2: Palm date spathe 2 mg/kg, PDS20: Palm date spathe 20 mg/kg, PDS200: Palm date spathe 200 mg/kg

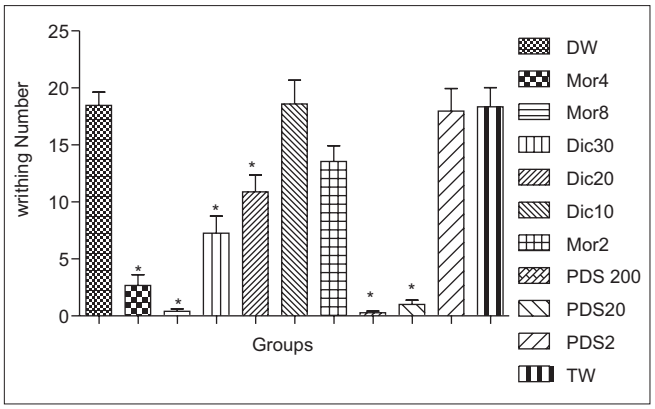

Figure 2: The effects of palm date spathe extract and positive control drugs on the number of writings as compared with control groups (n = 8). Based on the one-way ANOVA followed by Tukey's *post hoc* test, *indicate the significant differences, as compared with the control groups (P < 0.01). DW: Distilled water, TW: 20% Twin 80, Mor2: Morphine sulfate 2 mg/kg, Mor4: Morphine sulfate 4 mg/kg, Mor8: Morphine sulfate 8 mg/kg, Dic10: Diclofenac 10 mg/kg, Dic200: Diclofenac 20 mg/kg, Dic30: Diclofenac 30 mg/kg, PDS2: Palm date spathe 2 mg/kg, PDS20: Palm date spathe 20 mg/kg, PDS200: Palm date spathe 200 mg/kg

diclofenac or 8 mg/kg morphine, but the number of writhes in this groups was considerably lower than those received 30 mg/kg diclofenac (29 ± 0.13 vs. 7.25 ± 1.57, P < 0.05).

DISCUSSION

The results of this study showed that no analgesia is possessed by the PDS extract in acute pain model, but the chronic responses to painful chemical stimulus in writhing test were dose dependently attenuated by PDS extract and its antinociceptive effect in 200 mg/kg was identical to 30 mg/kg diclofenac and 8 mg/kg morphine.

Pain induced by intraperitoneal injection of acetic acid and its transmission is complex and involves the interaction of both peripheral and central structures. Different neurotransmitter systems such as serotoninergic, catecholaminergic, cholinergic, dopaminergic, and opioidergic may contribute in the modulation of acetic acid-induced pain transmission in the central nervous system.[19] Peripherally, in addition to the direct stimulation of pain receptors, intraperitoneal injection of acetic acid also leads to the production of inflammatory substances such as type E prostaglandins which are responsible for pain and writhing responses.[19]

Camphor derivatives, which are among the PDS extract constituents, may be involved in its centrally acting anti-inflammatory and analgesic effects, via stimulation and subsequent blockade of the sensitivity of transient receptor vanilloid subtype I channels, which are abundantly expressed in nociceptive neurons.[12,13] Peripherally, Coumarin derivatives, another PDS extract constituents, can inhibit lipid production and enhance the removal of free oxygen radicals.[11-20] Following the tissue damage, similar to what occurs after intraperitoneal injection of acetic acid; the group E prostaglandins are produced in the abdominal cavity and help the process of inflammation and intensification of pain, through sensitizing the nerve endings to bradykinin, histamine, and other released transmitters.[21] Coumarin and its derivatives, 7-hydroxy Coumarin, inhibit the production of prostaglandins E through inhibition of the cyclooxygenase enzyme systems, and results in the reduction of inflammation and pain induced by acetic acid.[11] Beta sterols that are a form of phytosterols and are abundant in PDS extract were also introduced as anti-inflammatory agents, however, their effect was weaker than the anti-inflammatory effects of hydrocortisone.[22,23]

A research conducted in 2011, by use of formalin, hot plate, and writing tests revealed that Vitamin C, carotene, phytosterols, and calcium in the plant products were considered to induce a significant anti-inflammatory and anti-nociceptive effect.[24] According to the findings of another study in 2008, acetyl 2, pyronie, scopoletin (a kind of Coumarin) and alpha spinasterol (a kind of phytosterols) found in plant extracts, are involved in attenuation of glutamate-induced pain in rats, through the blocking of glutamate receptors as well as, by inhibiting the pro-inflammatory cytokines such as alpha tumor necrosis factor and type 1 leukotrienes.[25]

In an experimental study, anticonvulsant effect of 3–4 dimethoxytoluene, as one of the major components of PDS, was investigated in four different convulsing models in rodents and supposed that the anticonvulsant effects could be due toincreasing the gamma-amino butyric acid levels in the brain, which inhibits the construction of nitric oxide enzymes, N-methyl-D-aspartate glutamate receptors, or by suppressing the brain noradrenergic pathways.[26]

According to the suggested effects for different PDS extract constituents, the analgesic effect of dates spathe extract may be through the central pathways involved in acetic acid-induced pain modulation or via antioxidant effect of sterol and flavonoids constituents of the extract[27] which inhibit the production of inflammation-inducing factors.[28,29] Furthermore, structural changes in the central nervous system such as increasing the number and responsiveness of alpha-2-adrenergic receptors in the spinal cord may be effective in modulating pain sensation by the extract.[30]

CONCLUSION

The findings of this study confirmed the opinions of traditional physicians regarding the analgesic effects of the date spathe, and approved that this analgesic effect induced by 200 mg/kg PDS is almost comparable with the analgesic effects of 8 mg/kg morphine and 30 mg/kg diclofenac. Based on the analgesic effect of some bioactive compounds in the spathe extract, it can be concluded that its analgesic effect may be due to the camphor derivatives, Coumarin, and phytosterols existing in it.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Longo D, Fauci A, Kasper D, Hauser S, Jameson J, Loscalzo J. Harrison's Principles of Internal Medicine. 18th ed. New York: McGraw Hill Professional; 2012.
2. Katzung B, Master S, Trever A. Basic and Clinical Pharmacology. 11th ed. New York: McGraw Hill; 2009.
3. Naidoo V, Swan GE. Diclofenac toxicity in *Gyps* vulture is associated with decreased uric acid excretion and not renal portal vasoconstriction. Comp Biochem Physiol 2009;149:269-74.
4. Mirheydar H. Herbal information usage of plants in preventaion and treatment of diseases. Tehran: Tehran University Publication; 2003.
5. Rezaeisadrabadi M, Dashti RM, Anvari M, Falah-Tafti H, Zanbagh S. The evaluation of the analgesic effect of hydroalcoholic extract of solanum melongena in syrian mice using tail flick test. J Shahid Sadoughi Univ Med Sci 2011;19:490-500.
6. Zargari, A. Iranian medicinal plants. Tehran: Tehran University Publications; 1997.
7. Mokhtari M, Sharifi E, Moghadamnia D. Effect of alcoholic date spath on changes in testis tissue and hormones, FSH, LH and testosterone in male rats. IJBMS 2006;9:265-71.
8. Mikki MS, Al-Taisan SM, Abdul-Aziz A. Isolation of the chemical constituents of the spathe of date palm. Saudi Arabia: Al-Hassa Regional Agricultural Research Center; 1989. p. 244-98.
9. Vyawahare N, Pujari R, Khsirsagar A, Ingawale D, Patil M, Kagathara V. *Phoenix dactylifera*: An update of its indegenous uses, phytochemistry and pharmacology. Internet J Pharmacol 2009;7:4. Available from: http://www.ispub.com/IJPHARM/7/1/8733#. [Last cited on 2014 Sep 16].
10. Jahani A. Dates a fruit of life. Tehran: Agricultural Sciences Press; 2002.
11. Fylaktakidou KC, Hadjipavlou-Litina DJ, Litinas KE, Nicolaides DN. Natural and synthetic coumarin derivatives with anti-inflammatory/antioxidant activities. Curr Pharm Des 2004;10:3813-33.
12. Schenone S, Bruno O, Ranise A, Bondavalli F, Filippelli W, Falcone G, *et al*. O-[2-hydroxy-3-(dialkylamino) propyl] ethers of (+)-1,7,7-trimethyl bicyclo[2.2.1]heptan-2-one oxime (camphor oxime) with analgesic and antiarrhythmic activities. Farmaco 2000;55:495-8.
13. Xu H, Blair NT, Clapham DE. Camphor activates and strongly desensitizes the transient receptor potential vanilloid subtype 1 channel in a vanilloid-independent mechanism. J Neurosci 2005;25:8924-37.
14. Dashti-r MH, Vahidi AR, Panjalizadeh ME. Effect of *Phoenix dactylifera* spathe hydroalcoholic extract on chronic pain in mice. J Med Plants 2012;11:136-44.
15. Samsamshariat SH. Extraction, separation, identification and structure elucidation of various types of natural products. Iran: Mani Press; 1992. p. 14-6.
16. Heilborn U, Rost BR, Arborelius L, Brodin E. Arthritis-induced increase in cholecystokinin release in the rat anterior cingulate cortex is reversed by diclofenac. Brain Res 2007;1136:51-8.
17. Mesdaghinia A, Samiei F. Investigation of stress induced analgesia with or without cck receptor agonists and antagonists in mice suffering from neuropathic pain using hot plate test. J Shahid Sadoughi Univ Med Sci 2004;12:39-42.
18. Habibi-Asl B, Soltany R. Effect of ephedrine on morphine antinociception and tolerance induced to morphine antinociception in mice. Pharm Sci 2005;2:11-6.
19. Koyama T, Fukuda K. Nociceptin receptor antagonist JTC-801 inhibits nitrous oxide-induced analgesia in mice. J Anesth 2009;23:301-3.
20. Witaicenis A, Seito LN, da Silveira Chagas A, de Almeida Junior LD, Luchini AC, Rodrigues-Orsi P, *et al*. Antioxidant and intestinal anti-inflammatory effects of plant-derived coumarin derivatives. Phytomedicine 2014;21:240-6.
21. Oh YC, Jeong YH, Cho WK, Ha JH, Gu MJ, Ma JY. Anti-Inflammatory and Analgesic Effects of Pyeongwisan on LPS-Stimulated Murine Macrophages and Mouse Models of Acetic Acid-Induced Writhing Response and Xylene-Induced Ear Edema. Int J Mol Sci 2015;16:1232-51.
22. Yu S, Li S, Henke A, Welzel G, Li S, Cheng B, *et al*. Development of novel sterol-based Liver X Receptor agonists as therapeutics for inflammatory diseases (HUM1P. 307). J Immunol 2015;194 (1 Supplement):52.32.
23. Loizou S, Lekakis I, Chrousos GP, Moutsatsou P. β-Sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells. Molecular Nutrition And Food Research 2015;54:551-8.
24. Abdel-Moein NM, Abdel-Moniem EA, Mohamed DA, Hanfy EA. Evaluation of the anti-inflammatory and anti-arthritic effects of some plant extracts. grasas y aceites 2015;62:365-74.
25. Ribas CM, Meotti FC, Nascimento FP, Jacques AV, Dafre AL, Rodrigues AL, *et al*. Antinociceptive effect of the Polygala sabulosa hydroalcoholic extract in mice: Evidence for the involvement of glutamatergic receptors and cytokine pathways. Basic Clin Pharmacol Toxicol 2008;103:43-7.

26. Al-Taher AY. Anticonvulsant effects of 3, 4-dimethoxy toluene, the major constituent of Phoenix dactylifera L spathe in mice. Sci J King Faisal Univ (Basic Appl Sci) 2008;9:115-25.
27. Pujari RR, Vyawahare NS, Kagathara VG. Evaluation of antioxidant and neuroprotective effect of date palm (*Phoenix dactylifera* L.) against bilateral common carotid artery occlusion in rats. Indian J Exp Biol 2011;49:627-33.
28. Vyawahare NS, Pujari RR, Rajendran R, Khsirsagar AD, Ingawale DK, Patil MN. Neurobehavioral effects of Phoenix dactylifera in mice. J Young Pharm 2009;1:225-32.
29. Mohamed DA, Al-Okbi SY. *In vivo* evaluation of antioxidant and anti-inflammatory activity of different extracts of date fruits in adjuvant arthritis. Pol J Food Nutr Sci 2004;13:397-402.
30. Bezerra MM, Lima V, Girão VC, Teixeira RC, Graça JR. Antinociceptive activity of sildenafil and adrenergic agents in the writhing test in mice. Pharmacol Rep 2008;60:339-44.

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