Dose Dependent Anti-inflammatory Effect of *Ammi majus* Alcoholic Extract in Rat: Chronic Study

Shihab H. Mutlag*,1

*Department of Pharmacology and Toxicology,College of Pharmacy,University of Baghdad,Baghdad,Iraq.

Abstract

During treatment of inflammatory diseases, many conventional therapies (non-steroidal anti-inflammatory drugs) used to relief pain and inflammation. Chronic use of the intended drugs is frequently associated with serious side effect, which may lead to discontinuation of treatment . The efficacy and dose- response effect of ammi majus extraxt (2 , 4, 8 , 16, and 32 mg/rat) were assessed using formalin to induce paw edema in rats as a model of chronic inflammation respectively. In this study, 42 rats were used and allocated into 7 groups each containing 6 rats, representing control (Distilled water) , standard (piroxicam) and test extract (2 , 4 , 8 , 16 and 32 mg/rat of *Ammi majus* alcoholic extract). The test extract and control were given orally before induction of inflammation Paw edema was measured by using vernier caliper after 7 days for chronic inflammation. The result indicated that *Ammi majus* alcoholic extract significantly lower paw edema ($p<0.05$) compared to standard and control, while the dose 16mg/rat also lower the paw edema compared with other test groups but less compared with the dose 32mg/rat. In conclusion, *Ammi majus* alcoholic extract possess anti-inflammatory activity in animals model of chronic inflammation and the effect increased with increasing the dose.

Key words: *Ammi majus* alcoholic extract, Chronic inflammation , Dose-Dependent .

العلاقة بين الجرعة والتأثير للفعالية المضادة للالتهاب للمستخلص الكحولي للخلة الشيطانية في الجرذان : دراسة مزمنة

شهاب حطاب مطلك*،[1]

*فرع الأدوية والسموم ، كلية الصيدلة، جامعة بغداد، بغداد ، العراق.

الخلاصة

في علاج الأمراض الالتهابية هنالك الكثير من الادويه المستعملة التي تسكن الألم أو تقلل الالتهاب لكن الاستعمال المستمر لهذه الادويه خصوصا عند مرضى الالتهابات المزمنة يكون مصحوبا بآثار جانبيه قد تؤدي إلى قطع العلاج السبب الذي ادى الى البحث عن علاجات بديله متمثله بالمستخلصات الطبية النباتية التي لها مفعول العلاجات المصنعة وأثار جانبيه اقل إضافة إلى رخص ثمنها . الهدف من هذه الدراسة هو تقييم فعاليه الخله الشيطانيه في النماذج التجريبيه للالتهابات المزمنة في الحيوانات المختبريه وكذلك معرفة فيما اذا كانت فعالية العلاج تزداد بزيادة الجرعة أو لا.استعمل في هذه الدراسة ٤٢ جرذ مختبري وقسمت الى سبعة مجاميع في كل مجموعة ست جرذان لتقييم تأثير الخله الشيطانيه في كل نموذ ج من النماذج حيث تمت دراسة التأثير المضاد للالتهاب لجرع مختلفه للمستخلص ٢ملغم ،٤ملغم ،٨ملغم ،١٦ملغم و٣٢ملغم للجرذ عن طريق الفم وكذلك تم استخدام مادة البايروكسيكام والماء المقطر كنماذج قياس وسيطره اثناء التجربه . تم استحداث الالتهاب في نماذج الالتهاب المزمنة بحقن ٢% فورمالين تحت جلد كل جرذ على التوالي . تم تقييم الالتهاب المزمنة بعد ٧ ايام من استحداث الالتهاب وتم قياس نسبة تأثير العلاج بطريقة أداة قياس السمك.في هذه الدراسة أظهرت الجرعه ٣٢ ملغم للخله الشيطانيه فعاليه ذات فرق معنوي في تقليل الوذمه الناتجه من حقن الفورمالين في نموذج الالتهابات المزمنه على طول فترة التقييم وبتأثير موازي لتأثير الادوات القياسيه المستعملة والمتمثله بمضادات الالتهابات غير الستيرويديه (بايروكسيكام) واضهرت النتائج ايضا تأثير للجرعه ١٦ملغم في تثبيط الالتهابات المزمنه بتأثير اقل مقارنة بالجرعة ٣٢ ملغم حيث ازداد التأثير بزيادة الجرعة. ويمكن الاستنتاج من نتائج الدراسه بأن تأثير الخله الشيطانيه اضهر نتائج جيده في نماذج الالتهاب المزمنه وخصوصا في الجرعه ٣٢ ملغم وان مفعول العلاج يزداد بزيادة الجرعة.

الكلمات المفتاحية : المستخلص الكحولي للخلة الشيطانية ، الالتهاب المزمن ، تأثير زيادة الجرعة .

Introduction

Inflammation is an important physiological reaction which occurs in response to a wide variety of injurious (bacterial infection or physical trauma) ultimately aiming to perform the dual function of limiting damage and promoting tissue repair [(1)]. It requires the participation of various cell types expressing and reacting to diverse mediator along a very precise sequence [(2)]. The inflammatory response is often initiated by the activation of resident macrophage through pattern-recognition receptors: this triggers the sequential release of pro-inflammatory mediators such as eicosanoids, cytokines, chemokines and protease which derive leukocyte recruitment and activation [(3)].

[1] Corresponding author E- mail : shihab_hattab@yahoo.com
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Chronic inflammation is a process of prolonged duration (weeks or months to years) in which active inflammation, tissue injury and healing proceed simultaneously [4]. It is characterized by **a-** infiltration with mononuclear cells including macrophage, lymphocytes and plasma cells **b-**tissue destruction, largely induced by the product of inflammatory cells **c-** repair, involving new vessel proliferation (angiogenesis) and fibrosis[5].Resolution of inflammation (anti-inflammatory response) is an active process controlled by endogenous mediators that suppress pro-inflammatory gene expression and cell trafficking, induce inflammatory cell apoptosis and phagocytosis. An optional balance between pro- and anti-inflammatory response is required to prevent the highly detrimental effect of extensive, prolonged or unregulated inflammation[3].*Ammi majus* is ancient herbal remedy used in the treatment of various therapeutic conditions , asthma and angina[6], an infusion is used to calm the digestive system, while the decoction of the seed, taken after intercourse appears able to prevent implantation of fertilized ovum in the uterus[7] and the seed contain furancoumarins which stimulate pigment production in skin expose to bright light [6,7].*Ammi majus* contain Linear furancoumarins (xanthotoxin , bergabten , imperatorin and isioimpiellin) which inhibt human liver CYP450[8,9] simple coumarins induce number of enzymes like aldehyde reductase , glutathione S-transferase(GST), and NAD(P)H quinone oxidoredctase in the liver that are possible for detoxification of alfatoxin B1[10] and also contain flavoniods (quercetin and keampferol) which has antioxidant and anti-tumor activity[11,12]The present study was designed to evaluate the efficacy and dose response effect of *Ammi majus* alcoholic extract in experimental animal model of chronic inflammation.

Materials and Methods

The present study was carried out on 42 rats of both sexes weighing 250, selected from the Animal House of the College of Pharmacy, University of Baghdad. The animals were maintained on normal temperature, humidity and light/dark cycle. They fed standard rat pellet diet and had free access to water until the night of the day of investigation.The animals were allocated into seven groups 6 animals each as follows . The control group was administered with 2ml/kg distilled water orally by gastric gavage tube , the standard group was treated with 5mg/kg piroxicam intraperitoneally while the test groups was treated with either 2, 4, 8, 16 or 32 mg/rat orally respectively. Chronic inflammation was induced by injection of 0.1 ml of 2% formalin into sub planter area of the right hind paw of the rat [13]. All treatment were administered 30 minutes prior to formalin injection and continued for seven consecutive days. The increase in paw thickness was measured by vernier caliper method [14] before and seven days after induction of inflammation..

Statistical Analysis

All data were expressed as mean ±SEM. Comparisons between treated groups were performed by ANOVA and students t-test to evaluate the statistical difference. The P value < 0.05 was considered significant.

Results

The anti-inflammatory effect of *Ammi majus* alcoholic extract on chronic inflammatory model was illustrated in(table 1)and (figure 1). Treatment with piroxicam significantly reduce formalin induce paw thickness ($p<0.05$) compared to control . *Ammi majus* alcoholic extract 16mg /rat and 32mg/rat showed significant reduction in paw thickness ($p<0.05$) compared to control, while *Ammi majus* alcoholic extract 2mg, 4mg and 8mg/rat showed non-significant reduction in paw thickness($p>0.05$) compared to control .Both piroxicam and *Ammi majus* alcoholic extract 16mg/rat produced comparable effect on formalin-induced chronic inflammation while *Ammi majus* alcoholic extract 32mg/rat showed significant difference to that produced by control , standard and all other treated groups (2mg, 4mg, 8mg and 16mg/rat) which produced the greatest effect with increase the dose of *Ammi majus* alcoholic extract .

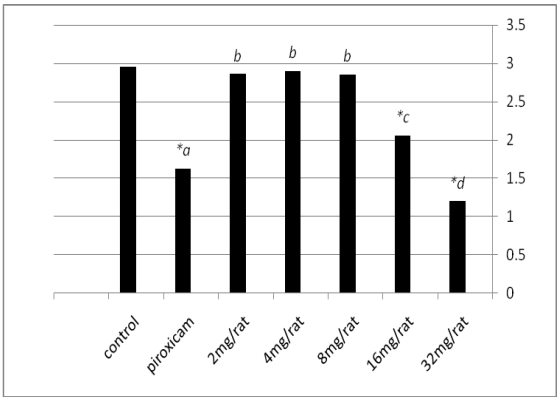

Figure 1: Mean increase in paw thickness during experimentally-induced chronic inflammation of the study groups.

Table 1: **Effect of different doses of *Ammi majus* alcoholic extract on formalin-induce chronic inflammation in rats.**

Treatment Groups	Mean increase in paw thickness(mm) after 7 days	% of inhibition
Control (2ml/kg D.W) n=6	2.96 ± 0.37	-
Piroxicam 5mg/kg n=6	1.63± 0.28^{*a}	16.11
Extract 2mg/rat n=6	2.86 ± 0.12^{b}	0
Extract 4 mg/rat n=6	2.9 ± 0.14^{b}	0.23
Extract 8 mg/rat n=6	2.85 ± 0.19^{b}	0.94
Extract 16 mg/rat n=6	2.06 ± 0.25^{*c}	12.79
Extract 32 mg/rat n=6	1.2 ±0.24^{*d}	24.88

Data are presented as mean ± SEM; n=number of animals; *P<0.05 with respect baseline value ; values with non-identical superscript (a,b,c and d) among different groups are considered significantly different (P<0.05).

Discussion

The inflammatory process is invariably characterized by a production of prostaglandins, leukotrienes , histamine , bradykinin , platelet-activating factor (PAF) and by a release of chemicals from tissues and migrating inflammatory cells[15]. The initial phase of inflammation (edema,0-1 hour) which is not inhibited by NSAID like indomethacin or aspirin , has been attributed to the release of histamine , 5-hydroxytryptamine and bradykinin [16], followed by a late phase (1-6 hours) mainly sustained by prostaglandin release and more recently has been attributed to the induction of inducible cyclooxygenase (COX-2) in the tissue[17]. Inflammatory events are initiated, enhanced, or coordinate by the action of various chemical mediators such as mast cells, platelets, and leukocytes are responsible for the release of inflammatory mediators and play an important role in the development of inflammation. These mediators include cytokines and chemokines , which promote inflammation and further function to amplify the response [18], low molecular weight lipids derived from arachidonic acid (AA) , gases like nitric oxide (NO) and carbon monoxide , reactive oxygen species (ROS) and nucleotides[19], small peptides such as kinins, complement and clotting system and finally amines such as histamine and 5-HT.Chronic inflammation begins 2-4 days after the onset of the acute response and can last for weeks to months or years due to the persistence of the initiating stimulus, interference of the normal healing process, repeated bouts of acute inflammation or low-grade smoldering due to continued production of immune response mediators[4] . The histological characteristics of chronic inflammation are the increase presence of macrophage and increased numbers of fibroblasts and other tissue matrix cells, such as osteoclasts and chondrocytes, via cytokine and growth factor-induced proliferation. Fibroblast is associated with secretion of both collagen and collagenase leading to fibrosis and reactive tissue remodeling. Phagocytic cell can also contribute directly to tissue injury through the release of proteolytic enzymes and free radicals [20].The effect of the of *Ammi majus* alcoholic extract on formalin-induced paw edema, as chronic inflammatory models was assessed by vernier caliper method. *Ammi majus* alcoholic (32mg/rat) significantly reduced paw thickness ($p<0.05$) and the level of inhibition was found to be higher than standard drug utilized in the study as shown in (figure 1) and (table 1) while *Ammi majus* alcoholic extract (16mg/rat) show less effect in paw thickness compared with piroxicam. *Ammi majus* alcoholic extract (2mg , 4 mg and 8mg/rat) showed no effect on paw thickness compared to other test groups .The anti-inflammatory effect of *Ammi majus* alcoholic extract may be explained by the many active constituents of the extract : *1(quercetin),* which is the most common flavoniod that scavenger both reactive oxygen species (ROS) and reactive nitrogen species (RNS). Consequently the flavonoid might be used to reduce both oxidative stress i.e an imbalance between the production of and protection against reactive species, and the inflammation. Moreover , querectin can also inhibit TF-kB activation, thereby directly reducing the cytokine production via this transcription factor[21]. Both these capacities of the intended flavonoid may contribute to the counteracting effect of quercetin on the lipopolysacchride(LPS) -induced tumor necrosis factor alpha(TNFα) . *2(kaempferol)* suppressed nuclear factor –kappaB(NF-kappaB) activating and expression of its target genes cyclooxygenase-2 inducible nitric oxide synthase , monocyte chemoattractant protein-1 , and regulate upon activation , and normal T-cell expressed and secreted in aged rat kidney. Furthermore , kaempferol suppressed the increase of the pro-inflammatory NF-kappaB

cascade through modulation of nuclear factor-inducing kinase (NK)/kappaB kinase(IKK) and mitogen-activated protein kinases (MAPKs) in aged rat kidney[22]. *3(coumarines)* like bergabten showed significant anti-inflammatory and analgesic activity; however xanthotoxin only have anti-inflammatory activity and isoimperatorin only analgesic effect . The anti-inflammatory and analgesic constituents seem to be related to the peripheral inhibition of inflammatory substance and to their effect on the central nervous system[23]. Imperatorin and isoimperatorin showed dual inhibitory activity due to their significant effect on 5-lipoxygenase and showed comparable inhibition on cycloxygenase1(COX1) and cycloxygenase2(COX2), when compared to indomethacin and nimesulide . Only imperatorin caused a significant reduction of nitric oxide (NO)generation[24]. Also psoralen , xanthotoxin have shown COX-2/5-LO dual inhibitory activity[25] . oxypeucedanin , imperatorin and isoimpertorin these compound have been reported to exhibit pharmacological effect such as inhibition of lipopolysaccharide-induced prostaglandin E2[26] , inhibition of IL-1B-induced cyclooxygenase-2 (cox2)[27] and inhibitory effects on the GABA degradative enzyme , GABA transaminase [28]. In conclusion , *Ammi majus* alcoholic extract in a dose dependant pattern was effective in decreasing chronic inflammatory reaction in experimental model, where the anti-inflammatory activity of *Ammi majus* alcoholic extract increase up to 32mg/rat and the effect increased with increasing the dose.

References

1. Nathan C. points of control in inflammation. Nature 2002; 420:846-852.
2. Gouwy M, Struyf S, Proost P, Van Damme J, Synergy in cytokine and chemokine network amplifies the inflammatory response. Cytokine Growth factor Rev 2005; 16:561-580.
3. Lawrence T, Willoughby DA, Gilroy DW. An ti-inflammatory lipid mediators and insights into the resolution of inflammation. Nat Rev Immunol 2002; 2:787-795.
4. Whicher J , Chambers R, Mechanisms in chronic inflammation . Immunol Today 1984;5:3-4.
5. Byrne AM. Anginogenic and cell survival function of vascular endothelial growth factor (VEGF)J Cell Med 2005;9:777.
6. Chevallier.A. The encyclopaedia of medicinal plants Dorling kindersly. London 1996; pp.450-621.
7. Bown D. Encyclopaedia of Herps and their Uses Dorling Kindersley. London . 1995; PP.560-748.
8. Cai , Y,-N., Bennet , D., Nair, R.V., Ceska, O., Ashwood – Smith , M. and DiGiovanni , J, Inhibition and inactivation of murine hepatic ethoxy and pentoxyresorufin activities by naturally occurring coumarins Chem. Res. Toxicol. 1993;6:872-879.
9. Cai, Y., Baer-Dubowaska, W., Ashwood-Smith, M.J, Ceska, O., Tachibana , S, and DiGiovanni , J, Mechanism based inactivation of hepatic ethoxyresorufin O-dealkylation activity by naturally occurring coumarins .Chem. Revs.Toxicol .1999; 9: 729-763.
10. Edenharder , R. and Tang , exhibition of mutagenicity of 2-nitroflourine , 3-nitrofluoranthene and 1-nitropyrine by flavoniod, coumarins , quinines and other phenolic compounds . Food Chem. Toxicol. 1997; 35:357-372.
11. Lin, J., Zhan, S.M. , Wu, K. , Willett , W. C , Fuchs, C.S. , and Giovannucci , E: Flavoniod intake and Colorectal Cancer in Men and Women :American journal of Epidemiology . 2006; 164:644-651.
12. Donnini , S. , Finetii, F, Morbidelli , L.L. , Cheneir , V., Barron , D. , Williamson, G. , Waltenberger, J. and Ziche. M,: Divergent effect of quercetin conjugate on angiogenesis: British Journal of Nutrition . 2006;95:1016-1023.
13. Chau TT. In pharmacological Methods in the control of Inflammations Alan R Liss Insc, New York, 1989;195-212.
14. Brownlee G. Effect of doxycortone and ascorbic acid on formaldehyde –induced arthritis in normal and adrenalectomized rats Lancet 1950;1:157-159.
15. Tomlinson A, Appleton I, Moore AR, Gilroy DW, Willis D, Mitchell JA,Willoughby DA. Cyclooxygenase and nitric oxide synthases isoforms in rat carrageenin-induced pleurisy .Br J Pharmacol 1994;113:693-698.
16. Salvatore C, Barbara P, Laura D, Angela I, Pasquale M. Rosiglitazone aligand of the peroxisome proliferator-activated receptor-g, reduced acute inflammation . Eu J Pharmacol 2004;483:79-93.
17. Nantel F, Denis D, Gordon R, Northey A, Cirino M, Mettes K, Chan C. Distribution and regulation of cyclooxygenase-2 in carrageenan-induced inflammation .Br J Pharmacol 1999;128:853-859.
18. Goldsby RA, Kindt TJ, Osborne BA, Kuby J . Immunology,4 th ed. New York: WH Freeman ; 2000.

19. Serhan CN. Lipoxins and aspirin triggered 15-epi-lipoxin are endogenous compounds of anti-inflammation : emergence of the counter-regulatory side. Arch Immunol Ther Exper 2001;49:177-188.

20. Copstead L-E, Banaski JL. Pathophysiology : Biological and Behavioral Prespectives . 2 nd ed . W.B. Saunders; Philadelphia: 2000.

21. Nair MP, Mahajan S, Reynolds JL, Aalinkeel R, Nair H, Schwartz SA and Kandaswami C.The flavoniod quercectin inhibits proinflammatory cytokine (tumor necrosis factor alpha) gene expression in normal peripheral blood mononuclear cells via modulation of the NF-kappabeta system .Clin Vaccine Immunol 2006;13:319-328.

22. Park MJ, Lee EK, Heo HS. et al. The anti-inflammatory effect of keampferol in aged kidney tissues : the involvement of nuclear factor –kappaB via nuclear-inducing kinase /l kappaB kinase and mitogen-activated protein kinase pathways . J Med Food 2009.

23. Chen, Y.F., Tsai, H.Y., Wu, T.S.: Anti-inflammatory and analgesic activities from the roots of Angelica pubescens. Plant Med 1995;61:2-8.

24. Abad , M.J., de las Heras , B., Silvan , AM., Pascual, R., Bermejo, P., Villar , A.M.: Effect of furancoumarins from Cachrys trifida on some machrophage function – J . Pharm. Pharmacol 2001; 53:1163-1168.

25. Kim , J.S., Kim , J C., Shim , S.H., Lee , E.J., Jin , W., Bae , K., Son , K.H.,K.H., Kim , H.P., Kang, S.S., Chang , H.W.: Chemical constituent of the root of dystaenia takeshimana and their anti-inflammatory activity –Arch.Pharm Res. 2006;29:617-623.

26. Ban HS, Lim SS , Suzuki K, Jung SH , Lee S, Lee YS, shin KH and Ohuchi K. inhibitory effect of furocoumarins isolated from the roots of Angelica dahuricae on prostaglandin E2 Production . plant medica 2003;69:408-412.

27. Lin CH, Chang CW, Wang CC, Chang MS and Yang LL. Byakangelicol, isolated from Angelica dahuricae, inhibit both activity and induction of cyclooxygenase - 2 in human pulmonary epithelial cells, Journal of pharmacy and pharmacology 2002 ;54:1271-1278.

28. Kim DK, Lim JP, Yang JH, Eom DO, Eun JS and Leem KH, Acetylcholinesterase inhibitor from the roots of Angelica dahuricae . Archives of pharmacia Research 2002;25:856-859.