Dose Dependent Anti-inflammatory Effect of Ammi majus Alcoholic Extract in Rat: Chronic Study
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Abstract
During treatment of inflammatory diseases, many conventional therapies (non-steroidal anti-inflammatory drugs) used to relieve 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 extract (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 (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.

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).

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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, inducing inflammatory cell apoptosis and phagocytosis. An optimal balance between pro- and anti-inflammatory response is required to prevent the highly detrimental effect of extensive, prolonged or unregulated inflammation (6). 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 furanocoumarins which stimulate pigment production in skin expose to bright light (6,7). Ammi majus contain linear furanocoumarins (xanthotoxin, bergabten, imperatorin and isioimpiellin) which inhibit human liver CYP450 (8,9) simple coumarins induce number of enzymes like aldehyde reductase, glutathione S-transferase(GST), and NAD(P)H quinone oxidoreductase in the liver that are possible for detoxification of aflatoxin B1 (10) and also contain flavonoids (quercetin and kaempferol) 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.](image-url)
Inflammatory cells act as sources of many mediators, which promote inflammation and further development of inflammation. These mediators and play a functional role in the development of inflammation. These mediators include cytokines and chemokines, which promote inflammation and further amplify the response. Low molecular weight lipids derived from arachidonic acid (AA), gases like nitric oxide (NO) and carbon monoxide, reactive oxygen species (ROS) and nucleotides, 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. 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. 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 : Il(quercetin), which is the most common flavonoid 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, quercetin can also inhibit TF-kB activation, thereby directly reducing the cytokine production via this transcription factor. 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

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

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

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. 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; 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. 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. Low molecular weight lipids derived from arachidonic acid (AA), gases like nitric oxide (NO) and carbon monoxide, reactive oxygen species (ROS) and nucleotides, small peptides such as kinins, complement and...
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 isomperatorin 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 isomperatorin showed dual inhibitory activity due to their significant effect on 5-lipoxygenase and showed comparable inhibition on cyclooxygenase1 (COX1) and cyclooxygenase2 (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 isomperatorin these compound have been reported to exhibit pharmacological effect such as inhibition of lipopolysaccharide-induced prostaglandin E2[26], inhibition of IL-1β-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


