Journal of Applied Sciences Research, 9(8): 5303-5312, 2013
ISSN 1819-544X
This is a refereed journal and all articles are professionally screened and reviewed

ORIGINAL ARTICLES

Anti-Inflammatory And Anti-Arthritic Activity Of Some Spices Extracts On Adjuvant Induced Arthritis In Rats

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ABSTRACT

Rheumatoid arthritis is the most common rheumatic disease and emerged as an important worldwide public health problem. The objective of the present research was evaluation of the anti-arthritic effect of thyme, turmeric, and cinnamon petroleum ether and ethanol extracts (300 mg/kg rat BW per day for 14 days) in adjuvant induced arthritis (AIA), which is similar to rheumatoid arthritis in human. The anti-arthritic effect and mechanism of action of these extracts have been studied through measuring the size of inflammation and determination of inflammatory and oxidative stress markers. The safety of the studied plants' extracts on liver and kidney functions were evaluated. Total phenolic, fatty acids and unsaponifiable matter (UNSAP) were assessed. Cinnamon showed the highest content of total phenolic compound (5.320 g GAE/100g sample). GLC investigation of UNSAP revealed that campesterol, stigmasterol and β-amyrin were present in all the studied plants. β-sitosterol was present only in cinnamon. GLC analysis of the fatty acids showed that linolenic acid (ω-3) was present in thyme, turmeric and cinnamon by 8.72%, 8.49% and 1.8%, respectively. All extracts produced significant reduction in inflammation ranged from 44 to 54%. Ethanol extract of turmeric showed the highest inhibition activity (54%). All studied extracts showed improvement in plasma malondialdehyde (MDA) level as oxidative stress marker and tumor necrosis factor-α (TNF- α) as inflammatory marker. Plasma levels of total protein, albumin and A/G ratio were increased significantly in arthritic rats given daily oral dose of extracts, while plasma globulin was reduced significantly. Oral administration of extracts showed significant improvement in nutritional parameters. All spices extracts showed also complete safety towards liver and kidney functions. The present study proves the anti-inflammatory activity of turmeric, cinnamon and thyme petroleum ether and ethanol extracts, which may have beneficial effects against rheumatoid arthritis onset/progression as shown in AIA rat model.

Key words: Anti-inflammatory, Adjuvant arthritis, Phenolic compounds, Cinnamon, Turmeric, Thyme.

Introduction

Rheumatoid arthritis (RA) is the most common rheumatic disease and emerged as an important worldwide public health problem (Boeing *et al*., 2012). RA affects about 1% of the world population. Women are affected three times more often than men (Theis *et al*., 2007). RA is a chronic inflammatory disease that primarily affects joints and causes pain, stiffness, swelling and limited motion (Joseph *et al*., 2010; McInnes and Schett, 2011). In RA the inflammatory process leads to progressive cartilage degradation with synovial hyperplasia, change in underlying bone with erosions and high levels of pro-inflammatory mediators (Belavic, 2010; Varani *et al*., 2010). It is widely accepted that cytokines such as tumor necrosis factor-α (TNF-α) and interleukin (IL) family mediate a large variety of effector functions in the context of RA pathogenesis (Emery and Dörner, 2011). The cause of the disease is unknown to a large extent. In addition to genetic factors, smoking, overweight, and nutrition contribute to the risk of disease (Pattison *et al*., 2004). As to nutrition, the risk seems to be increased by the consumption of red meat, protein, and coffee, while it is lowered by oily fish and olive oil. The adverse reactions and toxicity associated with the use of anti-inflammatory drugs have expeditiously promoted the use of natural plant products or procedures belonging to the diverse traditional systems of medicine by patients with RA (Chang *et al*., 2010) and other chronic inflammatory disorders (Alleva *et al*., 2010). This growing trend warrants a continuous search for new natural anti-arthritic products. Elevated oxidative stress might have an essential role in the etiology of rheumatoid arthritis, due to impaired antioxidant systems caused by reactive oxygen species (Kalpakcioglu and Senel, 2008). Reactive oxygen species have been implicated as mediators of tissue damage in patients **(**Winyard *et al.,* 2011**)**. Spices are one such source that has been used in cooking to add flavor and color to the food. A spice is a dried seed, fruit, root, bark, or flower of a plant. The use of spices has shaped a large part of the world's history. In ancient times, many spices were used as medicines for treating

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several diseases such as rheumatism, body ache, intestinal worms, diarrhea, hepatic diseases, urinary discharges, dyspepsia, inflammation, constipation, and dental diseases (Aggarwal *et al*., 2008, 2009). Spices are rich in antioxidants due to their phenol content these are able to block the formation of compounds that contribute to damage caused by metabolic disorders. Spices have very low calorie content and are relatively inexpensive; they are reliable sources of antioxidants and other potential bioactive compounds in diet (Muthulakshmi *et al*., 2009). The objective of the present research was evaluation of the anti-arthritic effect of thyme, turmeric, and cinnamon petroleum ether and ethanol extracts in adjuvant induced arthritis (AIA), which is similar to rheumatoid arthritis in human.

Materials And Methods

Materials:

- **Plant materials.** Turmeric rhizomes (*Curcuma longa* L., Family Zingiberaceae), cinnamon bark (*Cinnamomum xylanicum*, Family Lauraceae) and thyme aerial parts (*Thymus vulgaris* L., Family Lamiaceae) were purchased from local markets.
- **Chemicals.** Freund's complete adjuvant (FCA) (Sigma, USA) was used for induction of adjuvant arthritis in rats.
- **Animals.** Male Sprague Dawley rats 111-139 g were used in this study. Animals were kept individually in stainless steel cages at room temperature of 25 ± 2ºC and a relative humidity of about 55%; water and food were given ad-libtium.
- **Diets.** A balanced diet composed of 12% casein, 10% corn oil, 23.5% sucrose, 47% maize starch, 3% fiber, 3.5% salt mixture (Briggs and Williams, 1963) and 1% vitamin mixture (Morcos, 1967) was prepared for feeding rats all over the experimental period.

Methods:

- **Preparation of plant extracts.** The dried powder of different plants were separately placed in a continuous extraction apparatus (Soxhelt) and subjected to successive extractions using petroleum ether (40-60°C) then ethanol. The solvent of each extract was removed by evaporation under reduced pressure. All extracts were kept in deep-freeze till used.
- **Preparation of dosage form.** Ethanol and petroleum ether extracts of the turmeric rhizomes, cinnamon bark and thyme aerial parts were emulsified separately in water using gum acacia as emulsifying agent.
- **Determination of total phenolic contents in spices under investigation.** Total phenolics were determined colorimetric in the spices powder under investigation using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). Absorbance was measured at 765 nm using UVPC spectrophotometer. The total phenolic content was expressed as gallic acid equivalent (GAE) in grams per 100 gram.
- **Assessment of fatty acids, hydrocarbons and phytosterols contents of extracts.** The unsaponifiable fraction and fatty acid methyl esters of the studied extracts mixtures were prepared according to A.O.A.C (2000) and subjected to GLC analysis of fatty acids, hydrocarbons and phytosterols.

The unsaponifiable fraction was analyzed by GLC adopting the following conditions: Column: 10% OV-101 packed column; Stationary phase: Chromosorb W-HP; Detector temperature: 290°C; Injector temperature, 28°C; Carrier gas N_2; flow-rate 30 ml/min; air flow-rate: 300ml/min; H_2 Flow-rate 30ml/min; Detector FID; Chart speed: 0.5 cm/min; Oven program: Initial temperature, 70°C; Final temperature, 270°C; programmed 4°C/min. For 35min at 270°C, total time, 85 min. Identification of hydrocarbons and sterols contents of the unsaponifiable matter was carried out by comparison of their retention times with co-injected authentic reference compounds. Quantization was based on peak area integration.

Analysis by GLC of the methyl ester was carried out according to the following conditions: Stationary phase: 10% diethylene glycosuccinate (DEGS) packed column; oven temperature, 170°C; detector temperature, 300°C; injector temperature, 250°C; Carrier gas, N_2; flow-rate, 30ml/min; air flow-rate, 350ml/min; H_2 flow-rate, 350ml/min; detector, FID; Chart speed, 2cm/min. Identification of the fatty acid methyl ester was carried out by direct comparison of retention times of each of the separated compounds with authentic samples of the fatty acid methyl esters analyzed under the same conditions. Quantization was based on peak area integration.

Design Of Experimental:

- **Induction of rheumatoid arthritis in rats using adjuvant induced arthritis in rats (Studying Anti-arthritic effect).**

Forty-eight rats were divided into eight groups each comprised six rats. Six test groups were given daily oral dose 300 mg of ethanol extract of thyme aerial parts, turmeric rhizomes and cinnamon bark/kg rat body

weight or 300 mg of petroleum ether extract of thyme aerial parts, turmeric rhizomes and cinnamon bark/kg rat body weight. The other two groups served as control (one group as control normal and the second as control arthritic rats). A day after starting medication, arthritis was induced in all rats (except the control group which is a normal group) by subcutaneous injection of Freund's complete adjuvant into the subplantar region of the right hind-foot paw (Singh *et al*., 1992). All the oral medication continued for 14 days. Rats were maintained on the balanced diet all over the experiment. Paw thickness were measured before induction of arthritis and at the end of the experimental period using vernier calipers. At the end of experiment, the increases in the thickness of injected foot of rats of test groups were compared with that of the control arthritic rats. During the experiment, body weight and food intake were recorded. At the end of the experiment; total food intakes, body weight gain and food efficiency were calculated. After elapse of experimental period, rats were fasted 14 hours and blood samples were withdrawn from eye vein orbital of the anaesthetized rats. Plasma were separated for the determination of plasma uric acid **(**Watts, 1974), plasma TNF-α (an inflammatory biomarker) (Taylor, 2001) and MDA as indicator of lipid peroxidation and oxidative stress (Satoh, 1978). Plasma total protein (Rheinhold, 1953), albumin (Doumas *et al*., 1972) were determined as indicator of nutritional status. Plasma globulin and the ratio of albumin to globulin (A/G ratio) were calculated. The safety of plant extracts were studied through evaluation of liver and kidney functions. Plasma level of creatinine (Bartles *et al*., 1972) and urea (Fawcett and Scott, 1960) were determined as indicators of kidney function, while the activity of aspartate transaminase (AST), alanine transaminase (ALT) (Reitman and Frankel, 1957) were determined as indicator of liver function. To deduce the thickness of inflammation in rats of each group, the thickness of foot at the start of the experiment was subtracted from that at the end of the experiment.

- **Statistical analysis.** The results of animal experiments were expressed as Mean±SE and they were analyzed statistically using the one-way analysis of variance ANOVA followed by Duncan's test.

Results:

Table 1 showed the contents of total phenolic as g gallic acid equivalent/ 100g dry weight of different studied plants. Cinnamon showed the highest content of total phenolic compound (5.32 g GAE/100g) followed by turmeric (3.34 g GAE/100g) and thyme herb (3.04 g GAE/100g).

Table 1: Total phenolic contents of spices under investigation.

Plants	Total phenolic (g gallic acid equivalent/100g)
Turmeric	3.34 ± 0.055
Thyme	3.04 ± 0.098
Cinnamon	5.32 ± 0.098

Each value represents the mean of three replicates ± SE.

Table (2) and (3) showed the fatty acids and unsaponifiable matters in the plants extracts, respectively. The results of total fatty acids analysis revealed that oleic acid was the major unsaturated fatty acid in turmeric 71.49%, while stearic acid was the major saturated fatty acid, in cinnamon 47.79%. Linolenic acid (ω-3) was present in thyme, turmeric and cinnamon by 8.72%, 8.49% and 1.80%, respectively. GLC investigation of the unsaponifiable matters showed the presence of campesterol and stigmasterol in all the studied plants. β-sitosterol was present only in cinnamon by 9.16%. β-amyrin was present in all the studied plants. Turmeric showed the highest content of β-amyrin (13.72%). Total phytosterol was ranged from 28.63% to 33.39% in the studied plants. Thyme showed the highest contents of hydrocarbon 30.95%, while cinnamon showed the lowest content of hydrocarbons 16.83%.

Table 2: Fatty acids contents of the different plants (as percentage of total fatty acids).

Fatty acids	Thyme	Turmeric	Cinnamon
C12 (0)	0.37	-	-
C14 (0)	1.93	-	-
C16 (0)	2.17	13.65	20.65
C18 (0)	7.35	-	47.79
C18 (1)	-	71.49	13.25
C18(2)	-	4.39	3.61
C18 (3)	8.72	8.49	1.8
C20 (0)	3.11	-	-
C22 (0)	2.24	-	-
C24 (0)	2.82	-	-
Total identified saturated fatty acids	19.99	13.65	68.44
Total identified monounsaturated fatty acids	-	71.49	13.25
Total identified unsaturated fatty acids	8.72	84.37	18.66

Inflammation Thickness Of Arthritic Rats:

The increase in foot thickness (Thickness of inflammation) of the control arthritic rats at the end of the experiment compared with that of rats given the different treatments are present in Table 4. Oral administration of plant extracts suppressed the swelling in the foot significantly. All extracts produced reduction in inflammation ranged from 44 to 54%. Ethanol extract of turmeric showed the highest inhibition activity (54%). Petroleum ether extract of cinnamon and ethanol extract of thyme showed the lowest inhibition (44%) of inflammation volume.

Table 3: GLC analysis of unsaponifiable matter of the different plants (as percentage of total unsaponifiable matter).

Hydrocarbon & sterols	Thyme	Turmeric	Cinnamon
Hydrocarbon:			
Pentadecane (C15)	-	0.582	0.913
Hexadecane (C16)	-	0.496	0.295
Heptadecane (C17)	2.823	1.034	0.449
Octadecane (C18)	-	1.695	1.122
Nonadecane (C19)	0.145	1.160	0.184
Eicosane (C20)	0.026	1.454	1.634
Heneicosane (C21)	0.015	4.042	0.857
Docosane (C22)	0.506	2.099	1.047
Tricosane (C23)	1.643	2.351	0.859
Tetracosane (C24)	2.292	2.396	2.074
Pentacosane (C25)	-	0.942	0.873
Heptacosane (C27)	11.899	7.838	4.049
Nonacosane (C28)	11.598	3.634	2.477
Phytosterols:			
Campesterol	23.919	5.259	5.196
Stigmasterol	7.818	14.410	11.738
β-Sitosterol	-	-	9.159
β-Amyrin	0.847	13.719	2.534
Total identified hydrocarbon	30.947	29.723	16.833
Total identified Phytosterol	32.584	33.388	28.627

Table 4: The increase in the thickness of the injected foot (after 13 days) of adjuvant arthritis induction of arthritic rats given a daily oral dose (300 mg/kg rat body weight) of methanol or petroleum ether extracts of plants under study.

Groups	Mean± SEM	% Inhibition of inflammation
Arthritic control	0.45^{a}±0.013	-
PE. ext. Thyme	0.242^{b}±0.015	46
Eth. ext. Thyme	0.25^{b}±0.013	44
PE. ext. Turmeric	0.225^{b}±0.021	50
Eth. ext. Turmeric	0.208^{b}±0.020	54
PE. ext. Cinnamon	0.25^{b}±0.026	44
Eth. ext. Cinnamon	0.23^{b}±0.028	48

In each column the same letter means non-significant difference, while different letters mean significant difference at 0.05 probability. The data are expressed as mean values ± standard error.

Biochemical and Nutritional Parameters of Arthritic Rats:

The results of biochemical changes of arthritic rats given different treatments are shown in Table 5. Plasma level of uric acid, globulin, MDA and TNF-α were significantly higher in arthritic control rats than control normal. Plasma level of TNF-α as an indicator of inflammatory markers decreased significantly in arthritic rats given different plants' extracts compared to control arthritic but still significantly higher than normal control. Administration of different extracts showed significant reduction in plasma level of uric acid and MDA (indicator of lipid peroxidation and oxidative stress) with different degrees when compared with arthritic control but still significantly higher than normal control.

Plasma protein (Table 5) levels showed non-significant reduction in arthritic rats compared with normal rats. Plasma levels of albumin and A/G ratio reduced significantly in arthritic rats compared with normal control, while plasma globulin was increased significantly. Administration of different plants extracts significantly increased plasma albumin levels and A/G ratio with different degrees and reduced the elevation in plasma globulin.

Table 5: Plasma levels of total protein, albumin, globulin, and A/G ratio as indicator of nutritional status of normal and arthritic rats.

Groups	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	MDA (nmol/ml)	Uric acid (mg/dl)	TNF-α (pg/ml)
Normal	7.9^{a} ±0.071	4.1^{a}±0.071	3.8^{a}±0.046	1.1^{a}±0.026	8.4^{a} ±0.441	1.14^{a}±0.053	20.2^{a}±0.585
Arthritic control	7.65^{a} ±0.068	3.4^{b}±0.065	4.2^{b}±0.085	0.804^{b}±0.029	12.3b^{a}±0.601	1.84^{b}±0.051	34.5^{b}±0.803
PE. ext. Thyme	7.7 ^{a} ±0.105	3.9^{a}±0.058	3.8 ^{a}±0.095	1.04 ^{a}±0.034	9.58^{ac}±0.268	1.28^{ac}±0.042	28 ^{c}±0.516
Eth. ext. Thyme	7.7 ^{a}±0.087	3.8 ^{c}±0.156	3.9 ^{a}±0.181	1.01 ^{a}±0.093	9.31^{a}±0.432	1.16^{a}±0.077	27.5 ^{ed}±0.581
PE. ext. Turmeric	7.8 ^{a}±0.124	3.9 ^{a}±0.110	3.9 ^{a}±0.071	1.02 ^{a}±0.036	9.19 ^{a}±0.298	1.19^{a}±0.039	24.2 ^{c}±0.703
Eth. ext. Turmeric	7.8 ^{a}±0.096	4 ^{a}±0.093	3.8 ^{a}±0.079	1.04 ^{a}±0.039	8.40^{ac}±0.199	1.13^{a}±0.055	23.9^{c}±0.483
PE. ext. Cinnamon	7.9^{a}±0.137	3.9^{a}±0.085	3.9^{a}±0.098	1.01 ^{a}±0.031	9.25^{a}±0.253	1.24^{ac}±0.052	26.2^{d}±0.477
Eth. ext. Cinnamon	7.8^{a}±0.071	3.9^{a}±0.031	3.9^{a}±0.058	1.03^{a}±0.016	9.19^{a}±0.411	1.21^{ac}±0.21	28.5^{e}±0.764

In each column the same letter means non-significant difference, while different letters mean significant difference at 0.05 probability.
The data are expressed as mean values ± standard error.

Plasma levels of creatinine and urea as indicators of kidney function showed non-significant changes in all groups (Table 6). Also plasma activity of AST and ALT as indicators of liver function showed non-significant changes in all the studied groups. This revealed the safety of the studied extracts' (Table 6).

The results of nutritional parameters are shown in Table (7). Body weight gain, total food intake and food efficiency ratio decreased significantly in all arthritic rats compared to normal control. Oral administration of different extracts showed significant improvement in body weight gain, total food intake and food efficiency with different degrees compared with arthritic control. This improvement may be attributed to the improvement in food intake.

Table 6: Liver and kidney functions of different experimental groups.

Groups	AST (U/ml)	ALT (U/ml)	Urea (mg/dl)	Creatinine (mg/dl)
Normal Control	62.7^{a} ±1.021	15.45^{a}±0.178	28.2^{a}±0.734	0.751^{a}±0.018
Arthritic control	63.3^{a}±3.723	15.8^{a}±0.362	29.1^{a}±0.532	0.769^{a}±0.011
PE. ext. Thyme	62.2^{a} ±1.84	15.9^{a}±0.264	28.0^{a}±0.513	0.669^{a}±0.008
Eth. ext. Thyme	62.9^{a}±0.715	16.1^{a}±0.325	27.8^{a}±0.359	0.645^{a}±0.017
PE. ext. Turmeric	61.8^{a}±0.821	15.4^{a}±0.219	28.4^{a}±0.597	0.712^{a}±0.015
Eth. ext. Turmeric	63^{a}±0.731	16.2^{a}±0.268	27.9 ^{a}±0.848	0.713^{a}±0.012
PE. ext. Cinnamon	63.1^{a}±0.258	15.6^{a}±0.243	28.1 ^{a}±0.552	0.722^{a}±0.024
Eth. ext. Cinnamon	62.5^{a} ±0.619	15.7^{a}±0.243	28.6^{a}±3.81	0.725^{a}±0.025

In each column the same letter means non-significant difference, while different letters mean significant difference at 0.05 probability.
The data are expressed as mean values ± standard error.

Table 7: Nutritional parameters of different experimental groups (Mean± SE)

Groups	Initial body weight (g)	Final body weight (g)	Body weight Gain (g)	Total food intake (g)	Feed efficiency ratio
Normal control	123.3^{a}±2.108	163.5^{a}±2.045	40.2^{a}±1.778	224.7^{a}±3.0	0.179^{a}±0.006
Arthritic control	123.3^{a}±3.555	146.5^{b}±3.972	23.2^{b}±1.301	191.5^{b}±1.301	0.121^{b}±0.005
PE. ext. Thyme	123.2^{a}±2.937	151.3^{bc}±3.169	28.2^{c}±0.9	208.2^{c}±3.745	0.135^{c}±0.005
Eth. ext. Thyme	123.3^{a}±3.051	154.5^{bc}±2.061	31.2^{c}±2.675	210.5^{c}±2.675	0.147^{c}±0.010
PE. ext. Turmeric	123.2^{a}±3.081	152.2^{bc}±3.208	29.2^{c}±1.3	203.8^{c}±4.763	0.143^{c}±0.004
Eth. ext. Turmeric	123.3^{a}±2.498	152.5^{bc}±2.883	29.2^{c}±0.792	211.8^{c}±4.763	0.138^{c}±0.005
PE. ext. Cinnamon	123.3^{a}±2.847	151.2^{bc}±3.919	27.8^{bc}±1.301	206.5^{c}±5.9	0.134^{c}±0.004
Eth. ext. Cinnamon	123.2^{a}±2.508	155.3^{c}±3.7	32.2^{c}±2.197	212.7^{c}±3.711	0.151^{c}±0.013

In each column same letter means non-significant difference while different letter means significant difference at 0.05 probability.
The data are expressed as mean values ± standard error.

Discussion:

Nonsteroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs), and corticosteroids appear to be highly efficient drugs in the treatment of rheumatoid arthritis; but they may cause side effects that can range in severity from mild to serious. The major adverse drug reactions (ADRs) associated with NSAIDs is gastrointestinal ulceration or bleeding with effects on other systems. During their clinical use, gastrointestinal toxicities especially as upper gastrointestinal adverse events such as perforation, ulceration, and bleeding are reported in about 20% of patients taking long-term NSAIDs which are a major clinical limitation (Garella and Matarese, 1984). On the other hand, other arthritis therapies such as DMARDs and biological agents had risks of immune suppression and serious infection, respectively, with long-term usage. So researchers must try to find new anti-inflammatory and anti-arthritic agents or drugs with minimal side effects, the best sources for these agents are natural plants, herbs and spices. In the present research a spices extracts were evaluated as natural anti-inflammatory and anti-arthritic agents in adjuvant induced arthritis in rats.

Chronic inflammation is an important feature of many joint diseases, including rheumatoid arthritis (de Grauw *et al*., 2009). Adjuvant-induced arthritis (AIA) is an animal model of chronic inflammation which is similar to rheumatoid arthritis in human. AIA in rats is commonly used to evaluate compounds that might be of potential use as drugs for the treatment of rheumatoid arthritis and other chronic inflammatory conditions. It can be induced in rats by an intradermal injection of Freund's complete adjuvant (FCA); this injection into rats produces an immune reaction that characteristically involves inflammatory destruction of cartilage and bone of the distal joints with concomitant swelling of surrounding tissues. FCA induced a series of cellular events leading to T-cell activation and polyarthritis (Billingham, 1990). The inflammation associated with AIA is dependent on prostaglandin E2 generated by cyclooxygenases (COXs) (Billingham, 1983; Anderson *et al*., 1996). Besides, the role of cytokines likes tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) which have also been implicated in this model (He *et al*., 2003). AIA is characterized by inflammation, high oxidative stress and reduction in body weight (Ibáñez de Cáceres *et al*., 2000).

Induction of chronic inflammation in rats in the present work was reflected in the increased inflammatory biomarkers (TNF-α). Pro-inflammatory cytokines, including IL-6, IL-8 and TNF-α, are important cytokines in inflammation and are considered to be the most important mediators involved in the pathogenesis of RA (Brennan and Mclnnes, 2008). Previous studies have also discussed the role of cytokines in RA (Mclnnes and Schett, 2007; Brennan and Mclnnes, 2008). TNF-α has been shown to play an important role in RA and is known to mediate a variety of effector functions relevant to the pathogenesis of RA. In addition, therapies targeting TNF-α is also recognized as effective treatments for patients with RA (Chu *et al*., 2013). TNF-α was the biomarker of inflammation assessed in the present study, showed extreme elevation in control adjuvant arthritic rats compared to normal control. This result was in agreement with the work of Glenn and Kooyers (1966); Mahajan *et al*. (2007) and Al-Okbi *et al*. (2011). Oral administration of different extracts reduced TNF-α levels as a result of reducing inflammation. The anti-inflammatory action of studied spices extracts can be attributed to prostaglandin and/or cytokine inhibition. Rathi *et al*. (2013) reported that cinnamon bark polyphenol (CPP) (200 mg/kg for 10 days) showed significant reduction in elevated serum TNF-α concentration in the AIA model in rats. CPP demonstrated prominent action in animal models of inflammation and arthritis and therefore can be considered as a potential anti-rheumatic agent with disease-modifying action. Curcumin, an orange-yellow hydrophobic polyphenol which is a principal active component of turmeric, modulates the inflammatory response by down-regulating the activity of COX-2, lipoxygenase, and inducible nitric oxide synthase enzymes; and inhibits the production of the inflammatory cytokines TNF-α, IL-1, IL-2, IL-6, IL-8, and IL-12 (Goel *et al*., 2008). Intraperitoneal injection of turmeric extract containing 4 mg total curcuminoids/kg/day for four days prior to arthritis induction significantly inhibited joint inflammation in both the acute (75%) and chronic (68%) phases. Also oral administration of this extract to rats four days prior to arthritis induction, significantly reduced joint inflammation by 48 percent on the third day of administration (Funk *et al*., 2006).

The elevation in plasma levels of MDA in arthritic control rats in the current research is in agreement with the results of Tastekin *et al*. (2007) and Al-Okbi and Mohamed (2012). Administration of different spices extracts to arthritic rats produced significant improvement of inflammatory and lipid peroxidation biomarkers together with reduction of inflammation thickness.

Plasma urate has powerful antioxidant properties. At concentrations normally occurring in human plasma, urate directly scavenges hydroxyl radical, singlet oxygen and peroxyl radicals from lipid peroxidation (Heffner and Repine, 1989). Additionally uric acid prevents the oxidation of vitamin C (Sevenian *et al*., 1985). The present results showed significant increase of plasma uric acid in arthritic rats when compared with the normal control. Oral administration of different extracts reduced urate levels as a result of reducing of oxidative stress. In a previous study by Mohamed and Al-Okbi (2008), methanol and petroleum ether extracts of cinnamon and turmeric showed significant anti-gout effect through reducing urate levels in plasma and urine.

The present study showed non-significant changes in plasma protein. Plasma albumin and A/G ratio were reduced significantly in arthritic rats, while plasma globulin increased significantly in arthritic rats. Oral administration of different spices extracts improved these changes with different degrees. These changes of albumin, A/G ratio and globulin are a result of adjuvant arthritis. Adjuvant-induced arthritis is an experimental model of rheumatoid arthritis that causes anorexia and muscle wasting (Gómez-Sanmiguel *et al*., 2013). Plasma total protein was found to be affected by the inflammation and rheumatic diseases (Bradley, 1985). Ballentyne *et al*. (1971) found an increased catabolic level of albumin in rheumatoid arthritis in proportion to the rheumatic activity. Baum and Ziff (1979) also noticed an increased albumin breakdown in proportion to the activity of the rheumatoid arthritis. These results agree with the present results. The elevation in plasma globulin may be reflect to increase level of $\propto_1$-glycoprotein which reported by Hrycai *et al.* (1993). The significant decrease in the albumin to globulin ratio was apparent as a result of a significant decrease in the albumin along with the increase in globulin level. This result is in agreement with Takemura *et al*. (1996) who stated that low A/G ratio in infections or inflammatory status can be used as index of disease severity.

The anti-inflammatory effect of the studied spices may be attributed to the presence of polyunsaturated fatty acids, phytosterols and phenolic compounds. Polyunsaturated fatty acids, especially α-linolenic acid (ω-3), were present in all the studied plants. Polyunsaturated fatty acids of the ω-3 series are essential for normal growth and development. The health effects of these fatty acids include reduction of cardiovascular risk due to anti-arrhythmic, anti-inflammatory, anti-thrombotic and lipid lowering actions (Martain *et al*., 2009). Ingestion of ω–3 fatty acids decreases membrane arachidonic acid and concomitantly decreases the capacity to synthesize eicosanoids from arachidonic; eicosapentaenoic acid gives rise to the 3-series prostaglandins and thromboxans and the 5-series leukotrienes (Calder, 1998). Diets containing ω–3 fatty acids are beneficial for decreasing the levels of certain pro-inflammatory chemokines thereby delaying the onset of severity of autoimmune symptoms (Venkatraman and Meksawan, 2002). Alpha-linolenic acid can be converted to eicosapentaenoic acid, which competitively inhibits the oxygenation of arachidonic acid by cyclooxygenase. In addition eicosapentaenoic acid is able to act as a substrate for both cyclooxygenase and 5-lipoxygenase leading to inhibition of the inflammatory mediator prostaglandin E_2 and leukotriene B_4 (Calder and Yaqoob, 2009).

Phytosterols are important structural components of plant membranes and they play a key role in plant cell membrane function (Dillard and German, 2000). The present results showed the presence of β–sitosterol and stigmasterol in the unsaponifiable fraction of the studied plants. β-sitosterol, its glycoside and stigmasterol have been reported to have anti-inflammatory and immune-modulating activity (Bouic and Lamprecht, 1999; Gomez *et al*., 1999). Plant phytosterols display their anti-inflammatory activity through inhibition of secretion of interleukin-6 and TNF-α (Bouic, 2001). Phytosterols have been shown to possess antioxidant and anti-inflammatory activities (Mohamed *et al*., 2005).

Phenolic compounds are composed of several classes including flavonoids, anthocyanins, phenolic acids and catechins that are characterized by cyclic rings with hydroxyl substitutions at various positions (Duthie *et al*., 2000) which react readily with free radicals thereby preventing cell damage. Phenolic compounds have been reported to have multiple biological effects, including antioxidant activity and anti-inflammatory (Löliger, 1991).

Curcumin, a polyphenol isolated from turmeric, has been reported to have antioxidant and anti-inflammatory activity (Chen *et al*., 2006; Rahman *et al*., 2006) through inhibiting the generation of reactive oxygen species and nitrite radical (Joe and Lokesh, 1994) and down regulation of COX-2 and nitric oxide synthetase (Surh *et al*., 2001). The anti-rheumatic activity of curcumin has also been proved in clinical study (Deodhar *et al*., 1980).

Phenolic compounds in cinnamon have also been reported to have a marked antioxidant potential and to suppress lipid peroxidation (Lee *et al*., 2003; Ranjbar *et al*., 2006). Also ethanol extract of cinnamon barks have potential value as an antioxidant substitute (Yang *et al*., 2012).

Thyme oil contains carvacrol, as a major component, which supresses COX-2 expression and activate PPAR-α and γ . PPAR γ-dependent suppression of COX-2 promoter activity was observed in response to carvacrol treatment. In human macrophage-like U937 cells, carvacrol suppressed lipopolysaccharide-induced COX-2 mRNA and protein expression, suggesting that carvacrol regulates COX-2 expression through its agonistic effect on PPAR-γ. These results may be important for understanding the anti-inflammatory effects (Hotta *et al*., 2010).

The decrease in body weight gain and food efficiency ratio in control arthritic rats compared to control normal was expected since it has been reported that rheumatoid arthritis is usually associated with loss of lean tissues, which contain most of the body's protein (Bistrian and Blackburn, 1983). The decrease in body weight gain in the arthritic control rats may be due to tissue destruction in adjuvant arthritic rats. Rates of protease-mediated degradation of muscle protein were accelerated without changes in protein synthesis in experimental arthritis (Fagan *et al*., 1987). Intracellular proteolysis of muscle proteins by lysosomal proteases is mediated by PGE_2 and later increased during inflammation (Fagan *et al*., 1987). Also the reduction of body weight gain may be due to wasting muscle in experimental arthritis, which seems to be due to enhanced protein breakdown by the ubiquitin–protesome proteolytic pathway (Lecker *et al*., 2004; Granado *et al*., 2005). It was noticed that food intake was also reduced significantly, which may share in the reduction of body weight gain. It has been also reported that rheumatoid arthritis is associated with anorexia (Bistrian and Blackburn, 1983). It was noticed that all treatments produced significant increases in body weight gain and food efficiency ratio. The significant improvement in body weight gain in arthritic rats is an indicator for improvement of the adjuvant arthritis (Glenn and Kooyers, 1966).

In Conclusion:

Ethanol and petroleum ether extracts of thyme, cinnamon and turmeric can be safely used as anti-arthritic natural product. The anti-inflammatory effect may be attributed to the presence of polyunsaturated fatty acids, phytosterols and phenolic compounds.

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