Antibacterial properties of "Accmus" mouth wash

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Abstract

Antimicrobial herbs can be used individually or in combination to prepare mouth wash which is healthier and safer than the synthetic ones. In this study a new "Accmus" herbal mouth wash was prepared and its antibacterial properties were evaluated. Alcoholic, boiled alcoholic and aqueous extracts of "Accmus" mouth wash were prepared from the bark of *Acacia arabica, Acacia speciosa* and root of *Calamus rotang* in combination by tincture and hot extract methods respectively. Alcohol content and pH were also determined. Antibacterial properties of the above extracts were also studied against *Staphylococcus aureus, Bacillus* sp of Gram (+)ve and *Pseudomonas aeruginosa, Klebsiella* sp of Gram (-) ve *in vitro* by using agar well diffusion method. This study showed that the alcohol content and pH of mouth wash preparations were in acceptable levels. Aqueous extract exhibited better antibacterial activity compared with alcoholic extract and had maximum sensitivity towards *Bacillus* sp and low towards *Klebsiella* sp. *Staphylococcus aureus* was only inhibited by all preparations of mouth wash. So the hot extraction method was efficient than the alcoholic extraction and this could be recommended with antibacterial properties rather than the alcoholic extract of mouth wash. Further study is needed for further purification and characterization of active constituents from various solvent extracts of mouth wash against oral diseases.

Introduction

Mouth wash or mouth rinse is a product used to enhance oral hygiene. Commercial brands of mouth wash contain synthetic and semisynthetic chemical substances such as thymol, methyl salicylate, menthol, chlorhexidine gluconate, methylparaben, hydrogen peroxide etc [1] and also include water and sweetness such as sorbitol, sodium saccharin [2]. Sometimes a significant amount of alcohol is added as the carrier for the flavour. Sodium benzoate is a common preservative in commercial mouth washes [3]. The risk of acquiring cancer rises almost five times for users of alcohol containing mouth wash who neither smoke nor drink [4]. Mouth washes containing cetylpyridinium chloride are also associated with loss of taste sensation and brown discoloration of teeth [4]. To overcome such harmful effect natural mouth washes are available in markets and are produced from plant based healthy ingredients such as organic aloe vera, peppermint, clove bud essential oils, perilla seed extract etc. The present study is to prepare a new "Accmus" mouth wash from the bark of *Acacia arabica*, bark of *Acacia speciosa* and root of *Calamus rotang*. *Acacia arabica* (Karuvel- "T") is a tree, becomes under family leguminosae. Its bark has medicinal properties, mainly used in oral diseases. Hence, it has 24-42% of tannin. *Acacia speciosa* (Kadduvakai – "T") becomes under family mimosaceae. Its bark decoction is being used in orodental diseases for gargle. Powder of root bark is used for bleeding. *Calamus rotang* is a climber one and it is classified under family palmae. In traditional medicine the root of *Calamus rotang* has been used against many oral diseases such as gum bleeding and aphthous ulcer in form of decoction for gargling [5,6]. The objective of this study is to prepare a natural new "Accmus" mouth wash and test its antibacterial activity against Gram (+) ve and Gram (-)ve bacteria.

Materials and Methods

Collection of plant materials

The plant *Acacia speciosa* was collected by the Unit of Siddha Medicine, University of Jaffna, Sri Lanka and it was identified based on herbarium records in the Department of Botany, University of Jaffna and other relevant materials [7,8]. And healthy bark was obtained, washed under running tap water, dried in sun shade for three weeks. Then ground into fine powder. Bark of *Acacia arabica* and root of *Calamus rotang* were also collected from local market and their characters were compared with herbarium records [7,8]. The above parts were washed under running tap water, dried in sun shade for five days and then ground into fine powder, by using electric blender. The powder was stored in air tight dark bottles at room temperature.

Preparation of mouth wash

"Accmus" mouth wash was prepared by two methods.

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Tincture method

25 g of each of the above herbal powder was mixed and mixture was soaked in 93.75 ml of 25% ethanol and 281.25 ml distilled water for two weeks under direct sun light with occasional shaking. The mixture was filtered through double layered muslin cloth and the filtrate (355 ml) was collected into a clean dried dark bottle.

Half of the above volume of the filtrate was boiled at 85°C for 30 minutes and poured into a clean dried dark bottle as boiled alcoholic extract [9].

Hot extract method

25 g of each of the above herbal powder mixture was mixed with 250 ml distilled water in a sterile beaker. It was heated at 50°C on hot plate for 6 hours continuously till the final volume of extracts reached as 150 ml. Then extracts were filtered through double layered muslin cloth and the filtrate was concentrated by heating. It was kept at 4°C until used for assay [10].

Determination of pH was determined by pH meter.

Determination of alcohol content

Alcohol content of mouth wash was determined by ebuliometer. Durability of mouth wash also noted based on its characters such as color change, (odour) smell formation, turbidity and change in viscosity.

Antibacterial assay

Culture preparation.

The bacterial isolates of *Staphylococcus aureus*, *Bacillus* sp from Gram positives and Gram negative *Pseudomonas aeruginosa, Klebsiella* sp were obtained from bacterial culture collection, Department of Botany, University of Jaffna for this study. Test organisms were stored on nutrient agar slants at 4°C and these were sub cultured before 24 hours of the experiment and incubated at 37°C. After the incubation a loop full of young bacterial inoculum was transferred into the 10 ml of sterile saline water (0.85%) in an aseptic condition. Inoculum concentration was estimated by haemocytometer and the number of cells per ml was adjusted to 10^6 cells by using tenfold dilution [11].

Determination of antibacterial activity

Nutrient agar medium was autoclaved and cooled to 40°C. The antibacterial assay was performed by agar well diffusion method [12]. 1 ml of test culture (10^6 CFU/ml) was inoculated into a sterile petridish with 20 ml sterile nutrient agar and mixed well and allowed to solidify. Then wells were made by using sterile cork borer (8 mm in diameter) on the surfaces of agar plates and were filled with 100 µl of each extracts using sterile Pasteur pipette. 100 µl of commercially available "Chlorhexidine digluconate" mouth wash was used as standard and alcohol and water were used as control. Then plates were incubated at 37°C for 24-48 hours. Antibacterial activity was determined by measuring the diameter of the clear zone around the well. The above experiment was repeated five times and the mean diameter of the zone of inhibition was calculated.

Results and Discussion

Table 1: Antibacterial activity of mouth wash extracts on test bacteria

Mouthwash extracts	*Mean zone of inhibition (mm)*			
	S. aureus (+)ve	*Bacillus sp* (+)ve	*P. aeruginosa* (-)ve	*Klebsiella sp* (-)ve
Alcoholic extract of mouth wash (Tincture)	12	-	-	-
Alcoholic extract of mouthwash after boiling (Tincture)	10	-	-	-
Aqueous extract of mouthwash	15	16	12	10
Chlorhexidine digluconate (Standard)	16	20	15	13

Zone of inhibition includes the diameter of the well (8mm in diameter). (-) No activity.

Table 2: pH and alcohol content of mouth wash extracts

Mouth wash extracts	*pH*	*Alcohol content (%)*
Alcoholic extract of mouth wash	4.5	18
Alcoholic extract of mouthwash after boiling	5.1	3
Aqueous extract of mouthwash	5.8	-

Out of five samples of alcoholic mouth wash, turbidity was observed after 8 months in two samples and 11 months in other three samples. Whereas in aqueous mouth wash, cloudiness and colour change were observed after 3 days. This indicated that the durability period of alcoholic mouth wash was higher (8-11 months) than that of aqueous mouth wash (2-3days) at room temperature. But aqueous mouth wash could be kept safe at 4°C for 6-8 months.

In commercially available mouth wash, alcohol content goes up to 27% and the pH ranges from 5-7 [13]. These two parameters were in acceptable ranges in newly prepared mouth wash (Table 2). Results also showed that aqueous extract of mouth wash containing natural ingredients, exhibited better antibacterial activity when compared to alcoholic extract. It had maximum sensitivity towards *Bacillus* sp, while it had low sensitivity towards the *Klebsiella* sp. Among the tested bacterial growth, *Staphylococcus aureus* was only inhibited by both preparations of mouth wash. All tested bacterial growth was inhibited by the aqueous extract of mouth wash and the positive control "Chlorhexidine digluconate". But alcohol alone (control) didn't inhibit the growth of any tested bacteria (Table 1). This is due to less alcoholic concentrations and the tolerance of test bacteria. Aqueous natural mouth wash showed greater antibacterial activity than alcoholic extracts of mouth wash. Hot extract method was highly efficient for the extraction of antibacterial compounds rather than tincture method. Long term use of alcoholic mouth wash is not preferable, because of the hazardous effects especially for children and causes dehydration in mouth [14]. Recently the possibility that the alcohol used in mouth wash acts as a carcinogen has been raised [15]. Even though the durability period of aqueous mouth wash was low at room temperature, it showed greater range of antibacterial activity against test bacteria and absence of alcohol. So this could be recommended rather than the alcoholic extract of mouth wash.

Further studies should be done clinically and test the effectiveness of this "Accmus" aqueous extract of mouth wash against oral diseases.

Conclusion

In both preparations of mouth wash pH and alcohol content were in acceptable level. *Staphylococcus aureus* growth was only inhibited by both mouth wash preparations. Hot extraction method was efficient than that of alcoholic extraction. Aqueous mouth wash showed greater antibacterial activity against test bacteria and it could be recommended with antibacterial activity rather than the alcoholic extract of mouth wash.

References

1. Goldberg S, Konis Y, Rosenberg. Effect of Cetylpyridinium chloride on microbialadhesion to hexadecane and polystyrene. *Appl Environ Microbiol* 1990: 1678-82.
2. Giertsen E, Emberland H, Scheie AA. Effects of mouth rinses with xylitol and fluoride on dental plaque and saliva. *Caries Res* (1999); 33(1): 23-31.
3. Lachenmeier DW, Keck WA, Sauermann A, Mildau G. Safety assessment of alcohol containing mouth washes and oral rinses. *SOFWJ* 2008; **134**: 70-8.
4. Farah C, McIntosh L, McCullough M. Mouth washes. *Australian Prescriber* 2009; **32**: 162-4.
5. National Institute of Science Communications. The Wealth of India, Council of Scientific and Industrial Research, Vol II, New Delhi 2001; 97.
6. Muthaliyar M. Kunapadam (Moolikaivakuppu). 1936; **I**: 621-3.
7. Pandey BP. Taxonomy of Angiosperms, S. Chand and Company Ltd, 6th edition, Ram Nagar, New Delhi, 1997; ISBN: 81-219-0932-5.
8. Kugathasan KS. Check List of Some Plants of Botanical and General Interest With Brief Descriptions, Field Workcentre, Thondaimannaru, Jaffna. 2004; Bulletin no: 16.
9. Melntyre A. Herbs For Common Ailment, Gaia Books Limited, UK. 1992; 13.
10. Chikitsai S. Sarabenthiravaiththiyamuraikal, Saraswathi-Mahal, Thanjavour. 1951; 89.
11. Nester W, Evans Roberts C, Pearsall N, Anderson G, Nester T. Microbiology. A human perspective, WCB/Mc Graw-Hill, 2nd edition, 1998; ISBN-0-697-28602-9: 86-96.
12. Lino A, Deogracious O. The in vitro antibacterial activity of *Annona senegalensis, Securidacca longipendiculata* and *Steanotaenia araliacea* – Ugandan medicinal plants. *Afr Health Sci* 2006; 6: 31-5.
13. Lachenmeier DW, Keck-Wilhelm A, Sauermann A, Mildau G. Safety assessment of alcohol-containing mouthwashes and oral rinses. *SOFW J* 2008; 134: 70-8.
14. Cole P, Rodu B, Mathisen A. Alcohol-containing mouthwash and oropharyngeal cancer: a review of the epidemiology. *J Am Dent Assoc* 2003; **134(8)**: 1079-87.
15. Weaver C. Mouthwash linked to cancer. The Daily Telegraph (News Ltd). 2009; http://www.news.com.au/dailytelegraph/story/0,22049,24896583-5001021,00.html. (retrieved 12 January 2009).

Anti rheumatic herbal compound drug Yi Shen Juan Bi (YJB) as selective cytokines target in rheumatoid arthritis

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Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory disease often resulting in increased morbidity, mortality and disability. Cytokines regulate a broad range of inflammatory processes that are implicated in the pathogenesis of rheumatoid arthritis. In rheumatoid joints, it is well known that an imbalance between pro and anti-inflammatory cytokine activities favours the induction of autoimmunity, chronic inflammation and thereby joint damage. During recent decades a better understanding of the pathogenesis of RA has led to the development of new strategies for disease control which have transformed the management of RA. However, none of them are effective in curing rheumatoid arthritis. Furthermore, the potentially greater efficacy of treatment with TNF antagonists comes at a cost that is too high for the majority of the world's population and with more side effects. In this review we discussed about effective compound herbal drug Yi Shen Juan Bi (YJB) derived from traditional medicine as cytokine target in rheumatology. According to our published research findings YJB significantly ameliorate symptoms and prevented severe arthritis development in rats. Our studies showed that YJB significantly reduced the production of IL-1β, IL-6 and TNF-α *in vivo* and *in vitro*. These data indicate that YJB may have the potential to regulate the immunomodulatory cytokines. So these herbal compound drugs are more effective in the treatment of RA. It is our hope that this kind of traditional drugs can be developed to become new pharmaceutical agents that can be used in cost and clinically effectively for people suffering from rheumatic diseases.

Introduction

Rheumatoid arthritis (RA) is a common, chronic disease, for which multiple pharmacotherapies are generally applied. RA is a prevalent condition often leading to a high burden of suffering in patients [1]. Conservative treatment is mostly symptomatic and often associated with adverse effects. Therefore, it is understandable that many RA patients seek complementary and alternative medicine (CAM) to manage their illness [1]. In the USA, about 60 to 90% of arthritis patients use CAM [2]. An Indian study reported that around 40%of RA patients use either Ayurvedic or homeopathic medicines or TCM alongside conventional medicines [3]. According to the World Health Organization (WHO), traditional herbal preparations account for 30-50% of the total medicinal consumption in China [4].

The suppression of auto immunity in RA can be observed either as the induction of cell cycle arrest, which slows down inappropriate or uncontrolled cell division, or as the induction of apoptosis in stressed cells. Some anti-inflammatory plant natural products have been found to be very effective regulators of the cell cycle of autoimmunity by targeting specific cell signaling molecules, leading to apoptosis or cellular senescence. Many anti-inflammatory plant natural products have molecular signaling targets that can be potentially employed for treatment of rheumatoid arthritis. A main feature of a number of anti-inflammatory plant natural products is their action on the suppression of autoimmunity by upregulating key signaling molecules like Bax, Bak, and Bid and the subsequent down regulation of expression of various other key signaling molecules such as NF-κB, Bcl-2, and activate the caspases, in the nucleus and/or cytoplasm, which eventually induces apoptosis of target cells. Also most plant natural compounds respond to inflammatory mediators including IL-1, 4, 6, 8, 10, 12, 13, 17, 18, 21, TNF-α, TGF-η, IFN-γ, VIP, iNOS, and cyclooxygenase-2, prostaglandin E2.In this review we discussed herbal medicine cytokines targets in rheumatology special regards to Yi Shen Juan Bi (YJB) [5-8] (Table 1).

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Table 1: Ingredients of Yi Shen Juan Bi (patent number: ZL200510040550) [5-8].

Ingredient	*Composition (g)*
Rehmannia glutinosa	262.5
Eupolyphaga sinensis	210
Angelica sinensis	210
Bombyx batryticatus	210
Herba epimedii	210
Herba erodii	262.5
Buthus martensi	31.3
Corydalis yanhusuo	210
Scolopendra subspinipes	31.5
Panax ginseng	210
Polistes mandarinus (stir-baking)	210
Cynanchum paniculatum	262.5
Rhizoma drynariae	31.5
Polygonum cuspidatum	262.5
Pyrola rotundifolia	31.5
Millettia reticulata	262.5
Zaocys Dhumnades (stir-fried with wine)	210
Humulus scandens	262.5
Rehmannia glutinosa (dried)	210

The cytokine network in rheumatoid arthritis

Cytokines regulate a broad range of inflammatory processes that are implicated in the pathogenesis of rheumatoid arthritis. In rheumatoid joints, it is well known that an imbalance between pro-and anti-inflammatory cytokine activities favours the induction of autoimmunity, chronic inflammation and thereby joint damage. However, it remains less clear how cytokines are organized within a hierarchical regulatory network, and therefore which cytokines may be the best targets for clinical intervention a priority. Analysis of cytokine mRNA and protein in rheumatoid arthritis tissue revealed that many proinflammatory cytokines such as TNF alpha, IL-1, IL-6, GM-CSF, and chemokines such as IL-8 are abundant in all patients regardless of therapy [9]. This is compensated to some degree by the increased production of anti-inflammatory cytokines such as IL-10 and TGF beta and cytokine inhibitors such as IL-1ra and soluble TNF-R. In rheumatoid joint cell cultures that spontaneously produce IL-1, TNF alpha was the major dominant regulator of IL-1. Subsequently, other proinflammatory cytokines were also inhibited if TNF alpha was neutralized, leading to the new concept that the proinflammatory cytokines were linked in a network with TNF alpha at its apex. This led to the hypothesis that TNF alpha was of major importance in rheumatoid arthritis and was a therapeutic target. This hypothesis has been successfully tested in animal models of, for example, collagen-induced arthritis, and these studies have provided the rationale for clinical trials of anti-TNF alpha therapy in patients with long-standing rheumatoid arthritis. Several clinical trials using a chimeric anti-TNF alpha antibody have shown marked clinical benefit, verifying the hypothesis that TNF alpha is of major importance in rheumatoid arthritis. Retreatment studies have also shown benefit in repeated relapses, indicating that the disease remains TNF alpha dependent [9].

Immunomodulatory and anti-arthritic potential of traditional medicine

Ayurveda, traditional Chinese medicine (TCM), and other traditional systems are today yielding their theoretical and experiential frameworks to investigation by modern scientific techniques, applied mainly for the purpose of illustrating the effectiveness of remedies that have been developed over the centuries. In this context, the underlying theoretical framework fades away, and the tested substances become the focus of a new international effort at preventive health care and disease treatment. Herbal formulas developed today rely on a combination of traditional and modern indications for the use of the medicinal materials [10].

Arthritis has been a recognized medical condition since ancient times, and the Chinese had developed numerous formulas for its treatment. Chinese herbal formulas were not specifically designed for either of the two major types of arthritis defined today. The basis for Chinese doctors differentiating arthritis into subgroups was not the microscopic details of the pathology. Instead, arthritis was divided into traditional medicine categories: hot and cold types, upper and lower body involvement, deficiency or excess syndrome, pain characteristics (such as variability and severity), and whether the site of the arthritis was fixed or "moving." Both rheumatoid arthritis and osteoarthritis fall under the heading of bi syndrome, a disorder of qi and blood circulation that leads to symptoms of pain, numbness, swelling, and stiffness [11]. Rheumatoid arthritis fits most closely those syndromes characterized by the Chinese as wind-damp invasion affecting the joints. Osteoarthritis more closely fits the syndrome of liver/kidney deficiency syndrome causing weakness and stiffness in the legs with painful joints. In China, syndromes similar to rheumatoid arthritis were an area of special concern, generating considerable literature on the subject, since the condition could arise suddenly and could rapidly become severely debilitating [11]. Osteoarthritis, on the other hand, tended to be lumped together with other disorders of aging, in which stiffness and pain, especially of the legs, was considered just one part of the gradual deterioration of body functions that occurs with old age. As such, it is usually not the subject of much discussion separate from antiaging therapies. The closest traditional Chinese medicine term to rheumatoid arthritis is fengshi bing which literally means wind-damp disease [12-13]. The wind and damp factors can complex with either cold or heat factors to yield arthralgia. Almost all of the traditional approaches apply to the complex involving cold factors rather than heat. Gout, which has some characteristics in common with arthritis, usually fits the cold-dominated category or the cold-damp category of bi syndromes [12].

Chinese researchers have attempted to elucidate how the herbs used in traditional arthritis formulas alleviate the symptoms-from the modern viewpoint-by carrying out numerous studies of the blood constituents of patients [13]. According to studies that have been carried out recently the mechanism of action that may be dominant in the situations with good therapeutic results is a reduction in the levels of pro-inflammatory cytokines, such as interleukin-1 (IL-1), TNF etc [13]. The effect is then to alter the levels of T-cells and the production of activated antibodies and other components. In addition, or as a result, the properties of the blood and its circulation also change, with lowered sedimentation rate and improved circulation to the extremities. The herbs may also act on the prostaglandin synthesis and degradation pathways, yielding a lower level of pro-inflammatory prostaglandins [13].

YJB as selective cytokine targeted anti rheumatic drug

The past studies evaluated anti-arthritic potential of YJB in vivo and invitro rat models, which is very close to its human counterpart. In these studies, we used adjuvant arthritis (AA) induced and collagen induced experimental rat models for our experiments. One of the most imperative features of these models is chronic synovitis, including inflammatory cell infiltration, panes formation, and destruction of cartilage and bone erosion. According to our research findings YJB significantly ameliorate symptoms and prevented severe arthritis development in rats [5, 6, 7, 8, 14].

To elucidate the effect of YJB on immunomodulatory cytokines such as TNF-α, IL-1β and IL-6, an ELISA assay was performed. TNF-α and IL-1β are considered key mediators in the joint inflammation and in the destruction of cartilage and bone in patients with RA [14].TNF-α is a pivotal mediator in inflammatory arthritis including RA [15]. TNF-α is an autocrine stimulator as well as a potent paracrine inducer of other inflammatory cytokines such as IL-1β and IL-6. The blockade and inhibition of TNF-α reduces the production of other inflammatory cytokines in RA patients [16]. IL-6 is a proinflammatory cytokine with a wide range of biological activities in immune regulation, inflammation and oncogenesis [17]. IL-6 is known to be responsible for the increase of serum g-globulin and the emergence of rheumatoid factors [18]. High levels of IL-6 have been observed in both sera and synovial fluids from the affected joints of patients with RA [19]. Our studies showed that YJB significantly reduced the production of IL-1β, IL-6 and TNF-α [5-8].These data indicate that YJB may have the potential to regulate the immunomodulatory cytokines. Further our studies clearly confirmed that anti-arthritic property of YJB substantiated lower TNF-α, IL-1 production capacity of macrophages in *in vitro* [5].

The contribution made by proinflammatory cytokines in RA, such as tumour necrosis factor TNF-α and IL-1 has been validated in preclinical animal models and in humans [20]. It is also well documented in human RA and in animal models that IL-1 and TNF-α synergistically mediate synovitis and destruction of cartilage and bone [21]. TNF-α is an important regular factor in inflammation and immunity response, which can stimulate the synoviocyte and cartilage cells to synthesize the PGE2 and collagenase causing synovium and cartilage destruction as well as those of IL-1, IL-6, and IL-8 [22]. Therefore it is one of the most important factors in the cytokine network. In RA patients, IL-1β is overexpressed in inflamed synovial tissue, in particular in the lining layer and in sublining cells [23] and it is elevated in draining lymph from affected joints [20]. Furthermore cartilage from arthritis patients' exhibits upregulation of IL-1β mRNA as compared with normal cartilage [24, 25]. In addition, increased production of IL-1β in fibroblast like synoviocytes of susceptible individuals may lead to a higher risk of developing severe joint damage even in the absence of systemic inflammation. In general, TNF-α causes early joint swells in RA, while IL-1β combining with the immune complex leads to the cartilage erosion [25].

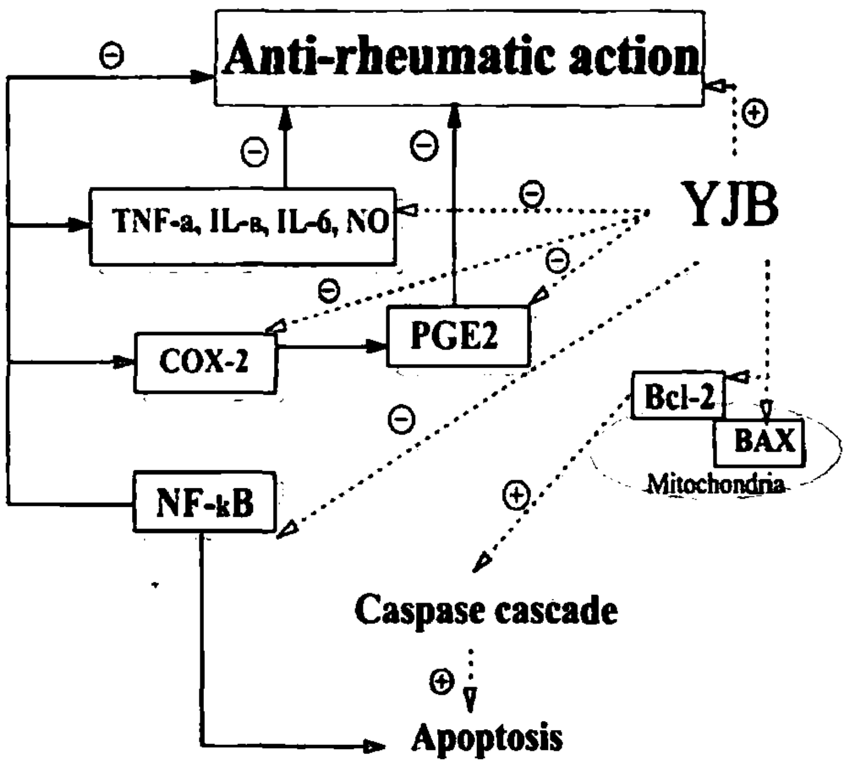
Considering these investigations it can be concluded that TNF-α and IL-1β have a pivotal role in the pathogenesis of RA. Furthermore based on these views, it can be pointed out that blocking of both TNF-α and IL-1β is necessary in the treatment of RA.

The study revealed that TNF-α mRNA and IL-1β mRNA expression in synovial cells of model group was significantly higher than that of normal rats. These are correlated with above research findings in RA. Furthermore pro-inflammatory cytokines, such as tumour necrosis factor α and interleukin-1β, are expressed in the arthritic joints in both AA rats and human rheumatoid arthritis, and blockade of these molecules results in amelioration of disease [26]. Our results confirmed that YJB could significantly decrease the TNF-α mRNA and IL-1β mRNA expression in synovial cells. This may be the one of the underlying mechanism that how YJB ameliorate inflammation in RA. Therefore it is a promising drug for the treatment of cytokine expression *in vivo*.

Conclusion

Taken together, our past results suggested that YJB can be effectively applied to inflammatory and immune diseases at the level of proinflammatory cytokines and mediator regulation (see proposed mechanism of YJB's activation.in Figure 1).

Figure 1: The mechanisms of YJB activation proposed here in scheme summarizes that the active components of YJB down regulate TNF-α, IL-β, IL-6, NO, PGE2, NF-κB and COX-2 expression, which results in enhance anti inflammatory and immunomodulatory action. YJB potently induces the apoptosis of synovium, via ultimate executioner caspase 3. YJB also down-regulates cytochrome-c related Bcl-2 expressing and up-regulates Bax expressing leading to triggered apoptosis cascade, which results in enhance apoptosis in the RA synovium and potentially limiting disease progression.'+': positive effects '-': negative effects 'solid-line arrow': known actions 'dotted-line arrow': our researches discovered actions [5-8].



References

1. Amarasinghe APG, Park J, Dwivedi M, Ernst E. Ayurvedic intervention for rheumatoid arthritis: a systematic review. *Focus on Alternative and Complementary Therapies* 2003; **8**: 526.
2. Soeken KL, Miller SA, Ernst E. Herbal medicines for the treatment of rheumatoid arthritis: a systematic review. *Rheumatology* (Oxf) 2003; **42**: 652-9.
3. Sangha O. Epidemiology of rheumatic diseases. *Rheumatology* (Oxf) 2000; **39** (Suppl 2): 3-12.
4. World Health Organization: Traditional medicine (accessed date: 25th August 2011). [http://www.who.int/mediacentre/factsheets/fs134/en/].
5. Perera PK, Peng C, Xue L, Li Y, Han C. *Ex vivo* and *in vivo* effect of Chinese herbal pill Yi Shen Juan Bi (YJB) on experimental arthritis. *J Ethnopharmacol* 2011; **134**: 171-5.
6. Perera PK, Peng C, Li Y, Fang W, Han C. Immunomodulatory activity of a Chinese herbal drug Yi Shen Juan Bi (YJB) in adjuvant arthritis. *Indian J Pharmacol* 2010; **42**: 65-9.
7. Perera PK, Peng C, Xue L, Li Y, Fang W, Han C. Effects of Yi Shen Juan Bi (YJB) pill on experimental rheumatoid arthritis. *Chinese Journal of Natural Medicines* 2010; **8**: 57-61.
8. Peng C, Perera PK, Li Y, Teng Q, Han C. Experimental study on secondary affection of rat's collagen arthritis treated with Yi Shen Juan Bi pill. *Chinese Journal of Traditional Medical Science and Technology* 2010; **17**(5): 389-90.
9. Iain B, Schett M, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. *Nature Reviews Immunology* 2007; **7**: 429-42.
10. Hsu HY, Hsu CS. Commonly Used Chinese Herb Formulas with Illustrations, rev. ed., Oriental Healing Arts Institute, Long Beach, CA. 1980.
11. Hsu HY. Neuralgia, rheumatism, and gout and their Chinese herb treatment. *Bulletin of the Oriental Healing Arts Institute* 1979; 4(4): 16-27.
12. Vangermeersch C, Sun Pei-lin. Bi-Syndromes, 1994 SATAS, Belgium.
13. Guillaume G, Chieu M. Rheumatology in Chinese Medicine, Eastland Press, Seattle, WA. 1996.
14. Ding CH, Li Q, Xiong ZY, Zhou AW, Jones G, Xu SY. Oral administration of type II collagen suppresses proinflammatory mediators production by synoviocytes in rats with adjuvant arthritis. *Clin Exp Immunol* 2003; **132**: 416-23.
15. Firestein GS. Evolving concepts of rheumatoid arthritis. *Nature* 2003; **423**: 356-61.
16. Kitamura K, Nakamoto Y, Kaneko S, et al. Pivotal roles of interleukin-6 in transmural inflammation in murine T cell transfer colitis. *J Leukoc Biol* 2004; **76**: 1111-7.
17. Bainbridge J, Madden L, Essex D, Binks M, Malhotra R, Paleolog EM. Methionine aminopeptidase-2 blockade reduces chronic collagen-induced arthritis: potential role for angiogenesis inhibition. *Arthritis Res Ther* 2007; **9**: R127-R127.
18. Naka T, Nishimoto N, Kishimoto T. The paradigm of IL-6: from basic science to medicine. *Arthritis Res* 2002; **4** (Suppl 3): S233-42.
19. Kim MJ, Kim HN, Kang KS, Baek NI, Kim DK, Kim YS, Jeon BH, Kim SH. Methanol extract of Dioscoreae Rhizoma inhibits pro-inflammatory cytokines and mediators in the synoviocytes of rheumatoid arthritis. *Int Immunopharmacol* 2004; **4**: 1489-97.
20. Dinarello CA. The IL-1 family and inflammatory diseases. *Clin Exp Rheumatol* 2002; **20**: S1-S13.
21. Choy EH, Isenberg DA, Garrood T, Farrow S, Ioannou Y, Bird H, Cheung N, Williams B, Hazleman B, Price R,

Yoshizaki K, Nishimoto N, Kishimoto T, Panayi GS. Therapeutic benefit of blocking interleukin-6 activity with an anti-interleukin-6 receptor monoclonal antibody in rheumatoid arthritis: a randomized, double-blind, placebo-controlled, dose-escalation trial. *Arthritis Rheum* 2002; **46**: 3143-50.

22. Choy EH, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. *N Engl J Med* 2001; **344**: 907-16.

23. Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, Smolen JS, Weisman M, Emery P, Feldmann M, Harriman GR, Maini RN. Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumour Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. *N Engl J Med* 2000; **343**: 1594-602.

24. Olszewski WL, Pazdur J, Kubasiewicz E, Zaleska M, Cooke CJ, Miller NE. Lymph draining from foot joints in rheumatoid arthritis provides insight into local cytokine and chemokine production and transport to lymph nodes. *Arthritis Rheum* 2001; **44**: 541-9.

25. Nadiv O, Beer Y, Goldberg M, Agar G, Loos M, Katz Y. Decreased induction of IL-1β in fibroblast-like synoviocytes: a possible regulatory mechanism maintaining joint homeostasis. *Mol Immunol* 2007; **44**(12): 3147-54.

26. Alten R, Gram H, Joosten LA, van den Berg WB, Sieper J, Wassenberg S, Burmester G, van Riel P, Diaz-Lorente M, Bruin GJM, Woodworth TG, Rordorf C, Batard Y, Wright AM, Jung T. The human anti-IL-1β monoclonal antibody ACZ885 is effective in joint inflammation models in mice and in a proof-of-concept study in patients with rheumatoid arthritis. *Arthritis Res Ther* 2008; **10**(3): R67-R67.