1 Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease in which body's immune system mistakenly attacks on joints, this causes inflammation that lines the inside of joints (Synovium) to thicken, resulting in swelling and pain in and around joints[(1)]. Inflammation associated with synovial membrane is primary cause for initiation of RA and bone erosion with uncontrolled inflammatory process leads to deformity of the joints[(2)]. Pathogenesis and etiological accounts, on RA characterizes involvement of joint swelling, tenderness, and destruction of synovial membrane leads to severe disability and uncontrolled release of inflammasomes and immune responses culminating into premature mortality associated with extra articular manifestations[(3)].

On epidemiological account RA affects 1% of population worldwide with 0.5% to 1% prevention and in India; the RA prevalence has been estimated to be 0.7%. RA can occur at any age, but is most likely to show up between ages 30-50 years. Disease involves younger people, elderly people and females. **Females have two folding risks for the outbreak of illness, but the disease's extra-articular manifestations are more prevalent in men, causing impairment and mortality [(4-7)].**

In clinical prospects inflammation in synovial membrane of metacarpophalangeal joint (small joints of fingers and feet) is major causative factor for initiation of RA. Pain, swelling, loss of function and a morning stiffness (lasting more than one hour) are common complaints of patients suffering with disease. Fatigue, malaise, weight loss, fever and depression are other constitutional symptoms which precede the onset of RA in primary disease stage[(1)]. Radiography and MRI (magnetic resonance imagery) are used frequently to detect morphological alterations in the form of destruction and deformities in cartilage and bones[(8)]. At primary phase osteopenia, with erosion and narrowing of joint space and loss of articular cartilage is major concern and in later phases of disease these tools can be used to mark symmetric patterns of the disease in form of radial- ulnar deviations, swan-neck deformities as well as boutonniere deformities in fingers. As disease progression occur hematological markers such as C-RP, ESR, Anti- CCP (ACPA) and Rheumatoid Factor (RF) becomes major criteria for diagnosis and interventions[(9)]. In advanced stages detection of IL-6 and TNF-α are also advisable to set the therapeutic regimen. RA differs clearly from person to person and

disease occurrence in different genders is highly dependent on auto immune responses and genetic variants (HLA-*DRB*) present in patients[10, 11].

Several biomarkers involved in the initiation of disease and RA has very complex pathophysiology which popularized the disease to have unknown etiology[2]. In the line to decode the disease stimulators different theories and pathogenic pathways were estimated to target the biomarkers and protein molecules to find a suitable management of disease but still there is a struggle in setting a common regimen for all the patients[12]. The first and foremost challenge in RA is to settle down the inflammation before progression of innate immunity activation which in sequence, stimulates adoptive immunity and provoke the common pathways which crosslink the inflammation and immune responses to produce autoantibody production responsible for autoimmune insult[13].

The first evidence in the progression of RA is always inflammation, and several theories were given for this pathogenic condition. Here according to newer theory of biomedical science and ancient wisdom of Ayurveda, RA is a metabolic condition which arises due to altered gut micro biota which causes the damage in gut wall and infiltrates the protein molecules which are not fully degraded. These infiltrated unbroken proteins (caused by increases gut permeability) are were sensed as the antigen via primary body defense system and anti bodies are generated against these molecules (ACPA)[14, 15]. Moreover the inflammation in gut wall and erosion in intestinal wall increase the microbial activity (chemokine production) and if these pathogenic molecules reach to the joint (chemotaxis) and attack, leading to there are high chances of progression of RA. The increase in micro flora also contributes in this pathogenesis due to overproduction of lipopolysaccharide which works as an endotoxine and initiates Toll like receptor activation[16]. This process plays a vital role in initiating the cascade of events in the form of activation of Neutrophills and Macrophages which are the first guardian of the innate immune system. These cells have TLR epitopes on their surfaces which are responsible for the activation of primary defense mechanism. On activation of proinflammatory cytokines, activation of T cell B cell and lymphocyte activated which results in increased microbial burden, endotoxemia, gut permeability and cell infiltration followed by impaired immune responses. This process is mediated by an interdependent network of cytokines, prostanoids, and proteolytic enzymes. Proinflammatory cytokines such as interleukins, Tumor Necrosis Factor-alpha (TNF-α) and NF-κB are central mediators in RA.

Being a lifelong disease, RA has a major concern of both morbidity and mortality due to involvement of the other organs; Lung, renal, heart and skin, which are major contributors in worsen the conditions of the RA patients[17].

The prevalence of cardiovascular complications (CVD) in patients suffering from Rheumatoid arthritis (RA) is one of the major concerns today due to high number of mortality among young patients with this condition[18]. There are two main reasons of occurrence of CVD in RA: The first being inflammation driven by TLR receptor activation, works as underpinning factor in both the diseases and second one is the side effects and drawbacks of existing therapies[19]. According to the European League Against Rheumatism (EULAR) and FDA the drug regimen (NSAIDs, DMARDs) of RA also contributes in progression of CVD[20].

The current study was oriented with two targets- [A] First target was to develop a validated rat model for problem statement and [B] Another target was to give a better therapeutic option for RA and its co morbid conditions.

However the therapeutic research for better treatment options in RA also have some limitations of recreating all the pathological pathways in single model at the same time which also suggests the open arms of this disease in better advancements. Also there is no such animal model developed till date which gives a complete insight of co morbid cardiovascular complications (Atherosclerosis in current study) in RA. The proposed hypothesis was aimed for development of animal models for (i) RA primarily by using Complete Freund's Adjuvant (CFA) and Bovine Collagen type II as inducing agents[21], as well as (ii) a model replicating CV complications in RA using Lipopolysachcharide (LPS) and High Fat Diet (HFD) with CFA and Collagen as secondary inducing agents.

Biological system when try to overcome these multiple events it releases markers like TNF-α, IL-6, IL-8, and NF-ƘB which act both as protective (when activated in required amount) and defensive (when overproduced). Here when LPS and HFD given to animals which are already challenged with CFA and collagen leads a physiological insult which triggers the highly expressed protein expression (TLR) in response to dual effects on synovium (leads RA) as well as on cardiac muscles (stimulates cardio vascular damages). This chain of event when continued it promote endotoxemia, cell infiltration and leaky gut responsible for cardiovascular complications with RA to represent human like pathogenesis in rat model of RA.[13]

To evaluate this preclinical and clinical resemblance clinical guidelines are already present in the form of European Alliance of Associations for Rheumatology (ELUAR) and American Association Rheumatology guidelines, but there were no validation criteria were reported for the same which recommended the validation of this novel model developed with four inducing agents on three basic criteria for model validation- (1) Face validity which represents the similarity in biology and symptoms between the animal model and human disease graded for core symptoms; Paw volume, Arthritic score, Arthritic index, X Ray, histopathology. (2) Constrict/Target validity- The target under investigation should have a similar role in the disease model as in clinical situation[22] graded for disease similarities and human resemblance; pain, symmetrical secondary lesions, digestion of digits, steatosis, Homocysteine levels and co morbidities and (3) Predictive validity-Clinically effective interventions demonstrate a similar effect in model graded for pharmacological parameters Neutrophil count, CRP, ESR, Anti-CCP, IL-6, TNF-α, RF Factor.

After this prevalidation the final comparison was done using questionnaire based validation system divided equally in selected eight domains for epidemiology, symptomatology and natural history criteria (SNH) matching with human disease onset, biochemical validation, pharmacological validation, histological validations, endpoint validation and genetic validation[23]. Each and every model was analyzed on this same weight score statistics using questionnaire framed for all the models and final score was analyzed for insufficiently validated, moderately validated and highly validated categories to identify the best suitable model for RA as well as RA with cardiovascular complications.

Being an auto immune disorder there are some problems in the current treatment therapy in arthritis. To overcome increased evidences of progression of disease different organization and committees in the field of Rheumatology as well as FDA are not mutually satisfied with the regimen or standards in treatment [20, 24]. Thus RA and its associated complications require serious thought process on possible alternatives which can substitute or overcomes the drawbacks of existing therapies.

Basic problem in the long term diseases like Rheumatoid Arthritis is continuation of same therapy over the period of time. According to FDA and ELUAR the therapies existing for disease prevention and management contains immunosuppressive agents, corticosteroids and NSAIDs which are known for their unavoidable side effects[20, 25]. This entire problem associated with disease and its management led us to search the better options for the management of the condition with common ingredients which can reduce medicine burden to the patient and easy to adhere with therapy which can be taken easily without side effects.

Moreover the progression of disease at initial stage can also be stopped by consuming this treatment with lesser side effects. On the above accounts when the validated model was developed, the treatment plan was designed to target RA and, CVD in RA (both the situation).

Traditional medication is one of the most accepted and adopted area from early ages. Each and every community and culture have evidences of use of plants and other natural components as medicinal drug and therapy. RA is one of the diseases which have more home remedy then the allopathic treatment. In Ayurveda and traditional Chinese and Indian medicines, *Curcuma longa, Boswellia serrata, Tripterygium wilfordil* and *Tanacetum parthenium* are some of the herbs which are accounted for relief against Rheumatoid Arthritis.[26] In present scenario commonly available Ayurvedic proprietary products have so many options in the form of ointments, Tailas and liniments to relive pain and inflammation. Maha Narayan Tel and Rheumasyl are most popular oils available in India for management of RA with local application. Therapeutic regimen in Ayurvedic marketed products includes purified herbs (Turmeric powder, Boswellia powder) and tablets. The promising usage of these medicinal herbs as anti-arthritic drug is expected to increase life expectancy and patient compliance in long term diseases. In such line of search, traditional anti-arthritic and anti-inflammatory properties of different common condiments and spices, came in to light viz; *Nigella sativa* seed*, Carica papaya* seed *and Momordica chirantia* seed are some of the traditionally used spices and condiments accounted as anti inflammatory and Immounomodulatory components which can be used as an additional therapy for prevention and maintenance of RA with the associated co morbid conditions.

Nigella sativa (NS) is one of the ancient medicinal plants having description in holy Bible and holy Quran as *seeds of blessing* which can cure many diseases[27]. *Nigella sativa* belongs to family of Ranunculaceae and commonly known as black seeds or kalonji, well known spice in Indian and Arabian cuisine. *Nigella sativa* is placed as an important therapeutic component in almost all traditional or indigenous systems of medicines which includes Ayurveda, Sidhdha, Unani and Tibb systems. Seed of *Nigella sativa* contains Thymoquinone as a major phytoconstituents possessing anti-inflammatory, anti-pyretic, anti atherogenic, anti tumor properties and *Nigella sativa* also have potential action against TNF-α, macrophage and neutrophil which generates pro inflammatory cytokine release[28, 29].

Carica papaya Linn (CP) seed commonly known as paw-paw seed is categorized as super food, flavanoids saponins, tannins, alkaloids, and anthraquinones are its major constituent

which is cardio protective analgesic, anti-inflammatory and wound healing properties. Traditionally these seeds are used for cardioprotective and immunomodulatory effect[30, 31].

Momordica chirantia (MC) also known as Karela or bitter guard is one of the commonly used vegetable. *Momordica chirantia* is member of Cucurbitaceae family and past research suggested the use of fruit having hypoglycemic and hypolipidemic properties. In the present study seed of bitter guard were taken for evaluating their anti inflammatory and anti arthritic activities. Phytochemical constituent present in seeds contains oil, and sterols possesses the anti bacterial anti microbial and anti inflammatory properties[32, 33].

All three seeds are chosen for the present study and aqueous extracts of these seeds were evaluated as treatment option due to their common pharmacological properties which synergistically acts as anti arthritic and Immounomodulatory drugs. As existing therapies for RA have risk associated with cardiovascular complications, this new synergistic combination evaluated to relieve this extra organ manifestation in the form of atherosclerosis along with existing Rheumatoid Arthritis in developed and validated model.

On the basis of above accounts *Nigella sativa, Carica papaya* and *Momordica chirantia* were selected as a treatment options for Rheumatoid Arthritis alone and along with cardiovascular complications.

Objectives of this study

1. **To compare *in- vivo* rat models for Rheumatoid Arthritis**
    1. Model I (CFA induced Rheumatoid Arthritis in rat)
    2. Model II (Collagen induced Rheumatoid Arthritis in rat)
    3. Model III (CFA + LPS induced Rheumatoid Arthritis in rat)
    4. Model IV (Collagen + LPS induced Rheumatoid Arthritis in rat)

    (*LPS was given in different doses to select suitable model)
2. **To develop and compare *in- vivo* rat models for cardiovascular complications in RA**
    1. Model I (CFA + LPS + High Fat diet induced Atherosclerosis in rat)
    2. Model II (Collagen+ LPS + High Fat diet induced Atherosclerosis in rat)

    (*LPS was given in different doses to select suitable model)
3. **To validate the developed models using same weight scoring method to select best models for both the models.**

4. **Pharmacological evaluation of aqueous extracts of *Nigella sativa, Carica papaya* and *Momordica chirantia* seed in validated model of RA at three dose levels.**
5. **Pharmacological evaluation of aqueous extracts of *Nigella sativa, Carica papaya* and *Momordica chirantia* seed in validated model of cardiovascular complications in RA.**
6. **To develop a formulation with selected combination of aqueous extracts of *Nigella sativa, Carica papaya and Momordica chirantia* seed.**