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A Review on the Pathophysiology and Screening Models of Rheumatoid Arthritis

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ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune systemic disorder caused by unknown etiology and characterized by chronic inflammation and synovial infiltration of immune cells. To find out exact mechanism behind the pathogenesis of this disease and evaluate the potential anti-arthritic drugs for clinical use, various animal models were developed. The appropriate animal models are selected depending upon two criteria's one is morphological similarities with human RA and second is the capacity to predict efficacy of various drugs in human. Various animal models like rat adjuvant arthritis, rat type II collagen arthritis, antigen induced arthritis, Proteoglycan induced arthritis, genetic models and In-vitro methods are discussed in this article. The induced models are developed for predicting efficacy of the anti-arthritic drugs. Various animal models are proven to be relevant to human rheumatoid arthritis. This helps in the development of various therapeutic regimens for the effective treatment and mitigation of the disease conditions associated with RA.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune and progressive inflammatory disease affecting 0.5 to 1% of adults worldwide. Women are more susceptible than men and the disease is more frequent at the age of 40–50 years. (1) Rheumatoid arthritis is characterized by synovial inflammation and hyperplasia, autoantibody production (rheumatoid factor and anti-citrullinated protein antibody [ACPA]), cartilage and bone destruction and systemic features, including cardiovascular, pulmonary, psychological, and skeletal disorders. RA leads to joint destruction and disability.

The joint damage can be prevented by diagnosis and therapy in the initial stages which leads to better long term results. Optimal management of RA is required within 3 to 6 months after onset of disease since substantial irreversible joint damage occurs within the first 2 years. Therefore, reliable biomarkers and outcome measures are required to establish early diagnosis, assess prognosis, and achieve better disease management. (5)

The cellular and soluble mediators of the immune system such as T cells, B cells, macrophages, cytokines, and prostaglandins are infiltrated in inflamed joints. These cells release proinflammatory cytokines such as interleukin IL-17, and tumor necrosis factor α(TNF-α) that play important roles in progressive joint destruction and are closely associated with the production of small proinflammatory lipid mediators such as prostaglandins. Animal models of rheumatoid arthritis are extensively used in research on pathogenesis of inflammatory arthritis and in the pharmaceutical industry in the testing of potential anti arthritic agents. The models explained here contributed to several current major advances in treatment.

PATHOPHYSIOLOGY OF RHEUMATOID ARTHRITIS

The etiology of RA is not known, but genetic and environmental factors are thought to cause autoimmune-directed pathological events. Antibodies that recognize endogenous proteins (i.e. autoantibodies) in the sera is an underlying characteristic of ~70% of RA cases, and autoantibodies can be present for years prior to RA diagnosis. Seropositive RA shows an increased risk for bone erosion and joint damage, while seronegative RA patients are often present with higher inflammatory activity. Rheumatoid factor (RF) directed against the Fc domain of IgG and anticitrullinated protein antibodies (ACPA) are binded to proteins, where

arginine is converted to citrulline which are the most prominent autoantibodies in RA and are useful for diagnosis.(11)

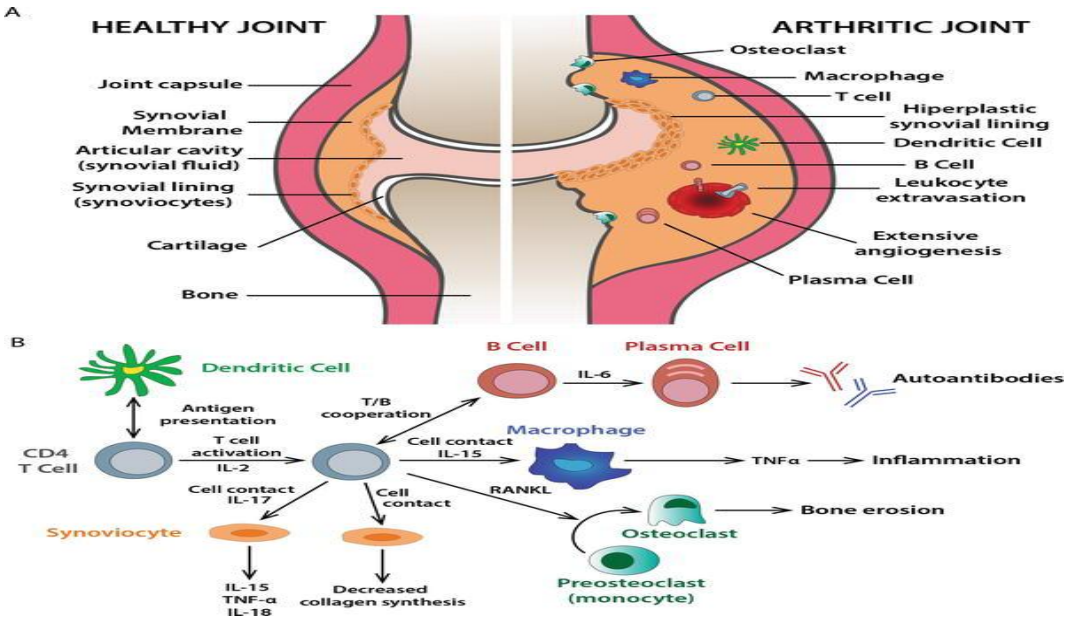

Figure No. 1: A- constituents of synovial membrane in healthy joint and arthritic joint B-Pathophysiology of production of autoantibodies, inflammation, bone erosion

ROLE OF AUTOANTIBODIES IN PATHOPHYSIOLOGY OF RA

The presence of autoantibodies is a characteristic feature of RA. Rheumatoid Factor and Anti-Citrullinated Protein Antibodies are the two most autoantibodies responsible for RA and provide different clinical and pathophysiological information. They are the cause for onset of disease symptoms and predict a more severe disease course. Therefore, disease management is efficient due to contribution of autoantibodies in the pathophysiology of RA. The hypothesis that autoantibodies may play a pathophysiologic role has been fuelled by the discovery of strong associations linking the HLA-DRB1 SE and PTPN22 alleles, smoking and the presence of autoantibodies, in particular ACPA.

RHEUMATOID FACTOR (RF)

Rheumatoid factor is the first autoantibody discovered in RA patients in 1948. It was called RF due to the high association with RA in 1952. Rheumatoid factor binds directly to the Fc portion of IgG and are produced by B cells present in Lymphoid follicles and germinal center like structures developed in inflamed RA synovium. RF is found in multiple immunoglobulin

isotypes (IgM, IgG and IgA) but IgM-RF is the one usually measured in most of the clinical laboratories, as they are being detected in 60–80% of RA patients. (5)

There is evidence that RF is a pathogenic autoantibody with a key role in the physiopathology of RA. Normally the transient production of low-affinity IgM RF is regularly induced by immune complexes and polyclonal B-cell activators. The physiological role in normal conditions of RF is to promote stability of IgG bound to surface of bacterial walls; enhances immune complex clearance by increasing its stability and size; helps B cells to uptake immune complexes and efficiently present antigens to T cells, and facilitates complement fixation by binding to IgG with immune complexes.

Due to high affinity and high-titre RF in RA synovial fluid, they potentiate inflammation and antigen trapping in the joints. In RA, RF induce the formation of immune complexes at the sites of synovial inflammation, activates the complement and leukocyte infiltration. B cells with RF migrates into the synovium of RA patients, providing a variety of antigens to T cells and this leads to local inflammatory responses and RF production in the synovium. Thus, RF prolongs B cell survival and hence maintaining its own production.

High titres of RF are associated with worse prognosis, aggressive articular disease, increased disease activity, and reduced rates of remission, higher prevalence of extra-articular manifestations, and increased morbidity and mortality, especially when in combination with ACPA.

Anti-Citrullinated Protein Antibodies (ACPA)

The autoantibodies reacting with citrullinated peptides (ACPA) was first reported in 1998. ACPA recognize peptides and proteins containing citrulline, a non-standard amino acid generated by the posttranslational modification of arginine by peptidyl arginine deaminase enzymes, in a calcium-dependent process known as citrullination. Post-translationally modified are capable of inducing immunological tolerance breakdown and autoantibody response. These modifications are critical for protein structure and biological function. Citrullination is seen during many biologic processes, such as inflammation, apoptosis and keratinization. The plasma cells in RA joints produce ACPA and the citrullination of proteins during the inflammatory process trigger autoantibody production. Many citrullinated proteins are found in RA synovium. While fibrin is the major citrullinated protein in RA joint. The development of arthritis is predicted not only by the presence of ACPA and also by their

levels in arthralgia patients. High titre ACPA recognizes several citrullinated epitopes. Patients with arthralgia, who have an extended ACPA repertoire are at higher risk for developing arthritis. (5)

Pathogenesis of ACPA

The identification of ACPA is the important breakthrough in understanding the pathogenesis in RA. An abnormal humoral response is exhibited to citrullinated proteins in RA patients in any form of inflammation in the synovium or elsewhere.

Under normal conditions, citrullinated proteins are regularly degraded and do not elicit any humoral reaction of the immune system, therefore the presence of citrullinated proteins per SE will not necessarily lead to chronic inflammation should be normally tolerated by the immune system.. Citrullination is seen in several physiological processes, such as cell death pathways, in which intracellular calcium concentration raises to higher levels than in physiologic conditions leading to activation of peptidyl arginine deiminases (PAD) enzymes during apoptosis. Inflamed tissue infiltered by immune cells contain PAD enzymes. PAD activated due to high intracellular calcium concentration during cell death promotes the citrullination of target antigens. Normally, the generated apoptotic bodies are rapidly removed by phagocytes, preventing inflammatory reactions.

Any irregulation of apoptosis involves the breaking of self-tolerance due to accumulation of dying cells and consequent accessibility of intracellular antigens. This intolerance promotes the meeting of citrullinated proteins with the immune system leading to autoantibody generation. This ultimately result in immune complex formation, followed by upregulation of proinflammatory cytokines, which is the driving force of the chronic inflammation that is typical of RA. (5)

Genetic factors, such as the HLA-DRB1 SE alleles, environmental factors, such as smoking and hormone levels, and the possible contribution of bacterial PAD enzymes might participate in this mechanism. The development of an autoimmune response against citrullinated epitopes is facilitated by specific genetic predisposition. The presence of particular HLADRB1 alleles (“shared epitope”-SE) in RA patients contributes to the development of anti-CCP antibodies. The presence of both RF and ACPA is associated with increased systemic inflammation and disease activity in RA. The combined presence of IgM-RF and ACPA mediates increased pro-inflammatory cytokine production. It is suggested that

IgM-RF enhances the capacity of ACPA immune complexes to stimulate macrophage cytokine production, therefore providing a mechanistic link by which RF enhances the pathogenicity of ACPA immune complexes in RA.

The key feature of RA is cartilage and bone damage in the form of erosions. Not surprisingly, substantial research has focused on the link between ACPA and activation of osteoclasts, the cells driving the erosive process in the bone. One scenario explaining this is indirect activation mediated by interaction of ACPA-containing immune complexes with FcγRs and release of TNFα. Besides that, it was hypothesized that ACPA could have an agonistic effect and enhance osteoclastogenesis by directly binding citrullinated proteins on the surface of osteoclasts.

The Role Of B Lymphocytes In RA Synovium

In recent clinical trials, B cells have been depleted by antibody treatment, which have shown efficacy as treatment for RA. In treated patients, the improvements are B cell depletion, decreased joint swelling and tenderness and reoccurrence of disease symptoms correlate to regeneration of B cells.

Some B cells leads to formation of immune complexes and complement deposition in the joints by producing autoreactive antibodies to cittrulinated proteins or anti-immunoglobulin rheumatoid factor. B cells are also sources of cytokines that leads to cellular activation, germinal center formation and inflammation in rheumatoid synovium.

The pathological role of B cells in RA is cell-cell interaction with T cells, dendritic cells, synovial nurse-like cells and fibroblasts. Lymphocytes are organized into complex structures called germinal centers (GC) in secondary lymphoid organs. At the center of the GCs are follicular dendritic cells surrounded by B cells. Surrounding the B cell-rich region is an area containing T cells and dendritic cells (DC) with a mantle zone for B and T cells to interact. The function of GC is to provide an antigen-specific cells to encounter APC bearing relevant antigens, thus, leading to signaling and activation through cell surface interactions and cytokine networks. Immunoglobulin genes in activated B cells undergo somatic hypermutation that leads to increased antibody affinity for target antigens and B cells differentiate within GC into plasma cells specialized to secrete antibodies. In addition, the GC provides an environment in which B cells may take up antigens and become more potent APCs by upregulating MHC and co-stimulatory molecule expression. (7)

Mechanisms of Bone Erosions in RA

The pathogenesis of focal bone erosions in RA relates to two aspects, the first one is the cell type responsible for bone resorption and the second one is the mechanisms that underlie the disturbance in bone remodelling that causes the progressive bone loss.

Under normal conditions, the signals for activation of the physiological bone remodelling cycle are initiated by osteoblast lining the trabecular bone surfaces. These cells within the adjacent bone marrow stroma and osteoblast receive the hormonal or cytokines signals that begin the remodelling cycle. The activated cells release additional cytokines and chemokines that are responsible for the recruitment and induction of osteoclasts responsible for bone resorption under physiological conditions.

The bone lining cells are required for formation of bone surface and are recognized by osteoclast precursor cells. The haematopoietic precursors from monocyte-macrophage lineage derived osteoclast precursor cells. These osteoclast precursor are present within the bone marrow or derived from circulating cells.

When the resorption phase is completed, the bone surface is repopulated with osteoblasts or preosteoblasts which are later converted into mature osteoblasts. These mature osteoblasts deposits on bone matrix that undergoes mineralization to form new bone surface. The osteoclasts and osteoblasts than undergoes apoptosis.

In physiological remodelling cycle, amount of bone removed during resorption phase must be equal to the amount of bone laid down during the formation phase. This remodelling helps the skeleton in adapting to changes occurred in biochemical environments and also in repairing any microdamage. The erosive changes occurred in RA with presence of inflammatory synovial lesion that are due to imbalance between bone resorption and formation. This imbalance leads to progressive focal articular bone loss.

MODELS COMMONLY USED FOR DRUG TESTING

INVIVO METHODS

Collagen-induced arthritis (CIA)

This is the most commonly used RA animal model. It is a Th1-mediated model that consists of both chronic inflammatory joint disease and autoimmunity targeting, which is major

feature of articular cartilage-type II collagen (CII). Rats are given id/sc injections of bovine type II collagen of 2 mg/ml in incomplete Freund's Adjuvantat the base of the tail on day 0 and day 7. Onset of arthritis disease occurs on days 10-13 and after the development of the disease, rats are randomized to study groups and treatment is proceeded. (14)

CIA primarily affects the peripheral joints that is the fore and hind-paws; however, the knees are rarely involved. The poly-arthritis is characterized by cartilage destruction with immune complexes deposition, bone resorption and periosteal bone proliferation with mild synovitis.

The histology of CIA resembles RA by the presence of infiltrating cells in synovial tissue and destruction of bone and cartilage. The inflammatory mediators and various proteolytic enzymes released within the joint space are due to infiltered neutrophils monocytes, locally activated chondrocyte and synovial cells that regulates the severity of CIA. Thus CIA is regulated by both the innate and adaptive immune systems(13). Susceptibility to CIA and development of RA is also linked to the specific class II major histocompatibility complex molecules. The immune system develops to maintains the inflammation in the CIA model. B cells and specific antibodies that recognize collagen, influences the pathology where T cells play a role in inducing the disease and supports the activated collagen-specific B cells in CIA rats(13). The arthritis was associated with sustained levels of serum anti-collagen antibody titres, higher levels of T-cell proliferation and IFN-g secretion in the late stage of disease.

Adjuvant-induced arthritis

Rat adjuvant arthritis is an experimental model of rheumatoid arthritis that is widely used for preclinical evaluation of various anti-arthritic agents which are either under preclinical or clinical investigation or current therapeutics of this disease. Induction of adjuvant arthritis is done with Freunds complete (FCA) supplemented with Mycobacterium (0.1 ml) in liquid paraffin is injected intradermally at one of the hind paw in the subplantar region of rats, it is used in the study of acute inflammatory reaction in the local area as well as immunological reaction that developed later in the paw and other organs after 9 days. Hindpaw swelling is monitored from day 9 when the onset of disease starts till day 15 or greater depending on desired duration. In the later stages of disease, adjuvant arthritis rats are often immobile due to severity of paw swelling. The symptoms of arthritis develops between 10-14 days.

The characteristic feature of this model is rapid onset, easily measureable, polyarticular inflammation, marked bone resorption and periosteal bone proliferation. Cartilage destruction

occurs but it is mild in comparison to the inflammation and bone destruction that occurs (14). The genetic background of rats is important, as both MHC and non-MHC genes contribute to their susceptibility to AIA.

The severity of the RA is permanent joint malformations, including ankylosis. Symptoms of AIA like joint swelling, lymphocyte infiltration, and cartilage degradation are commonly seen features with human RA (13). In AIA model, activated T cells are detected in the inflamed joints. T cells infiltrating the joint originate from various compartments; including the spleen, draining lymph nodes, Peyer's patches, and the recirculating T cell pool. The immune response is induced by specific antigen Hsp65, being the responsible epitope. Cytokines expressed in the joint during the early stages of inflammation include IL-17, IFN, and TNF-α, accompanied by cytokines involved in macrophage stimulation. As inflammation progresses in the joint, increased levels of IL-4, IL-6, monocyte chemotactic protein 1 (MCP-1)/CCL2, and TGF β can be detected. TNF-α, IL-1β, IL-21, and IL-17 are all involved in the pathology of this disease.(14)

Antigen-induced arthritis

Antigen-induced arthritis is seen in mice, rats and rabbits by intra-articular injection of protein antigen into the knee joints of animals that have previously immunised with the same antigen.

Any animal of choice is immunized either by subcutaneous or intradermal injections with the antigen which is a cationic substance such as methylated bovine serum albumin (m-BSA) which binds to negatively charged cartilage and will be retained in the joint. The antigen is then injected in one or both joints and acute inflammation which rapidly progress to joint destruction.

The pathogenesis involves an Arthus reaction on the articular cartilage as antibodies that binds to positively charged antigen is injected which further forms complexes that activate the complement locally and results in cartilage destruction. Mice have been extensively used to study efficacy of biologies and to attain various aspects in role of some specific cytokines in disease pathogenesis. (14)The cellular basis is very similar to CIA, but with more challenges and sensitisations that are being exploited. It is CD4+ T-cell dependent. The histopathological appearance of antigen-induced arthritis has similarities to RA, including synovial lining layer hyperplasia, perivascular infiltration with lymphocytes and plasma cells,

lymphoid follicles, pannus and cartilage erosions. Indeed repeated injections of antigen can induce ectopic lymphoid structures (ELS) similar in appearance to those seen in RA patient. However, antigen-induced arthritis is a monoarticular disease that affects only the injected joints. Antigen-induced arthritis is not susceptible to MHC class II and thus making this model useful for studies of transgenic and gene knock-out mice.

Proteoglycan induced arthritis (PGIA)

Proteoglycan isolated from human cartilage is used to induce arthritis in susceptible strains of mice. Among mice strains PGIA can be induced only in BALB/c and C3H mice and does not have MHC specificity. Development of poly-arthritis, deposition of immune complexes, and the presence of rheumatoid factor are similar features with human RA.

Proteoglycan, emulsified with an adjuvant is intraperitoneal (IP) injected on 0 and 21st day and also on 42nd day. Previously CFA was used as the adjuvant, whereas now-a-days dimethyl dioctadecyl ammonium bromide (DDA) is widely used in studies; as it offers the advantage of early onset of arthritis and increased severity of arthritis, without granuloma formation and tissue irritation, as seen with CFA. After injection of the emulsion, strong B cell and T cell responses develop. B cells have a dual role in PGIA as they function as autoantibody-producing cells and are crucial in their role as antigen presenting cells (APCs), and they activate proteoglycan-specific T cells. The adoptive transfer of arthritis to recipient mice requires both B cells and T cells in order to be successful; neither B cells nor antibodies alone can confer arthritis to recipient mice. It may be proposed that subsequently produced antibodies form immune complexes with proteoglycan in mouse cartilage locally in the joint, resulting in complement deposition and further immune cell activation as well as cytokine and chemokine production. Studies have shown that TNF and IL-1β are expressed during the effector phase of PGIA, as determined from messenger RNA (mRNA) isolated from joints. PGIA has been used less often than CIA, due to the fact that human cartilage has to be obtained and extensively processed in order to prepare the needed proteoglycan fraction.

GENETIC MODELS

Transgenic and knockout mice used as models of rheumatoid arthritis: The mice that is modified genetically has dual role for the study of arthritis. Deletion or introduction of genes for specific receptors, signalling molecules, cytokines, or other factors helps in testing the role of these genes in immunologic mechanisms. However spontaneous inflammation occurs

resulting in arthritis or any other inflammatory disorder. These models provide valuable information regarding the role of genes in inflammatory process and acts as a tool to study the effect of therapeutics in mice with joint inflammation.(13)

Spontaneous arthritis due to recognition of a ubiquitous self-antigen: the K/BxN model

Investigations indicate that arthritis pathways are developed in several mouse strains without administrating any external antigen, adjuvant, or antibody as seen in the K/BxN mouse with KRN T cell receptor transgenic mouse on the C57BL/6 x NOD background. This model develops chronic, progressive inflammation spontaneously. A visible joint inflammation is observed in 3 weeks of age which progress into a severe chronic inflammatory arthritis. T cell and B cells promotes joint destruction by secreting auto-antibodies. Nevertheless of MHC, the complement and Fc receptor role in this RA model is similar to CIA model. Thus, K/BxN model is considered as an important tool to study the role of antibodies in development of RA. This model also indicates that a joint-specific antibody is not required for inducing arthritis.(13)

Human TNF– transgenic mice

The human TNF– transgenic (Tg197) mice consists of high levels of soluble and transmembrane human TNF α that develops into spontaneous arthritis. At the age of 3-4 weeks, synovial hyperplasia and inflammatory cell infiltrates are observed. The mice fully develops the disease after 10 weeks of age. The similar features with human RA are synovial hyperplasia, the presence of an inflammatory cell infiltrate, pannus formation, bone resorption and cartilage destruction. Mice generated by crossing human TNF– transgenic mice with RAG-1–knockout mice have no T cells or B cells. Fibroblast-like synoviocytes in synovium of human TNF– transgenic mice induces synovitis, cartilage damage, and bone damage when transplanted into normal mice, further it illustrates that there is lack of involvement of immune cells in this model of arthritis. In this model, the effect of excess TNF and the relationship between TNF and IL-1 in the pathogenesis of arthritis is studied. The model is used very effectively in the assessment of anti-TNF therapies and also for testing other biologics and small molecules.(13)

INVITRO METHODS

Inhibition of protein denaturation

Denaturation of proteins as one of the causes of rheumatoid arthritis is well documented. Production of auto antigens in certain rheumatic diseases may be due to in vivo denaturation of proteins. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding.

Procedure

The reaction mixtures (0.5 ml) consisted of 0.45 ml\ bovine serum albumin (5% aqueous solution) and0.05 ml of plant extract and standard prednisolone. pH was adjusted at 6.3 using a small amount of 1 N HCI. The samples were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm7. For control tests 0.05ml distilled water was used instead of extracts while product control tests lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows:

Percent inhibition = [100 - (O.D. of test-O.D. of product control) / O.D. of control] x 100

EFFECT ON MEMBRANE STABILISATION / INHIBITION OF MEMBRANE LYSIS

Lysosomal enzymes that are released during inflammation leads to a variety of disorders which causes tissue injury by damaging the macromolecules and lipid peroxidation of membranes responsible for pathological conditions such as heart attacks, septic shocks, and rheumatoid arthritis etc. The extracellular activity of these enzymes is related to acute or chronic inflammation. Stabilization of lysosomal membrane in inflammatory response is by inhibiting the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, that may further causes tissue inflammation and damage upon extracellular release or by stabilizing the lysosomal membrane. Stabilization of human red blood cell membrane (HRBC) by hypotonicity induces membrane lysis which is used in in-vitro studies to measure the anti-inflammatory activity of the drugs or plant extracts.

Procedure

The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36 %], 0.5 ml HRBC suspension [10% v/v] with 0.5 ml of plant extracts and standard drug methotrexate of various concentrations (50, 100, 250, 500, 1000, 2000 µg/ml) and control (distilled water instead of hypo saline produce 100 % hemolysis) were incubated at 37ºC for 30 min and centrifuged respectively.

The haemoglobin content in the suspension was estimated using spectrophotometer at 560 nm.

$$\% \text{ of Inhibition} = 100 - [(\text{O.D of test solution}) \div (\text{O.D of control}) \times 100].$$

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