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1 INTRODUCTION

Worldwide plants have been playing a significant role in the treatment of various diseases and disorders of human being from centuries\textsuperscript{1}. Many of the plants are still being used as sources of medicines, in the form of source for pure compounds and intermediates for synthesis of new drugs\textsuperscript{1}.

In recent existence, people are in search of natural remedy not only to reduce the risk of various health conditions but also to treat or control the same\textsuperscript{1}. Different phytomedicines have evolved different phytochemicals, ingredients and enzymes as an antioxidant defense to maintain growth and metabolism as result of increased concern about improving health with a net result of advance research on plants\textsuperscript{2}.

Peoples have been using herbal medicines from millions of years and therefore plants or herbal medicines are an important source of drugs\textsuperscript{3}. Over these years there has been an enormous contribution of plants in the process of drug discovery, however many of the plants are still required to be explored for their usefulness\textsuperscript{4-8}.

This study investigates a parasitic leafless plant \textit{Cuscuta reflexa}, for its pharmacological, biochemical and toxicological effects on experimental animals. ‘Amarwel’ as the name indicates this climber is immortal and golden yellow colored ever growing stems indicating limitless source\textsuperscript{9}. The ability of the plant to absorb various constituents from host plants, its immortal nature and various traditional claims of the plant to be useful in various ailments like pain, inflammation and liver disease\textsuperscript{10} gave us an impetus to validate this
ethno pharmacological information. The plant is rich in phenolic constituents and contains various flavonoids; and hence it was decided to evaluate details of the effects exerted by this plant on various animal models of pain, inflammation, oxidative stress and helminthes infections.

Pain is a sensation which is difficult to define, it is an emotional incidence for any individual but generally it is any undesired sensation which is generated by virtue of damaging conditions such as tissue damage. Pain has been associated with many diseased conditions as it involves activation of nociceptive fibers. Both acute and chronic pain are related with tissue damage and inflammation and are frequently causing morbidity looking at the present status of drugs in clinical use there is an urgent need of safer and better analgesics and antiinflammatory drugs specially for the treatment of chronic pain and inflammation.

Oxidative stress and hepatotoxicity are other important clinical concerns in front of the society. Many environmental pollutants, along with some drugs are associated with the oxidative stress and are contributing in the pathogenesis of various diseases. Hepatotoxicity is of high concern worldwide despite of advancement of medicines; inadequacy of allopathic medicines for hepatotoxicity is prevailing and indicating towards the search for hepatoprotective herbal drugs.

To establish as a source for newer, safer and better drug, it was decided to test the usefulness of Cuscuta reflexa for these clinical conditions using scientific approach along with the available knowledge base.
1.1 Pain

Pain is a sensory stimulus that is unpleasant in nature and which is frequently related with the ruining of tissue which is also accompanied by emotional pattern consisting of anxiety and tendency to escape the feelings of nociception\(^3\).

The mechanism behind propagation of pain is called as nociception and the receptors involved are called as nociceptors\(^3\). These sensory receptors which are generally located in the periphery, are activated by any noxious stimuli like that induced by heat, pressure, irritation and once activated, they carry those impulses to the CNS\(^14\). These primary afferent nociceptors meet with others at Substantia gelatinosa of spinal cord to further transmit these pain sensations towards thalamus. The emotional aspects along with sensory experience are thought to be because of activation of this central pathway\(^3\).

However, some of the chemical mediators released in the proximity of nociceptors are also known to be associated with pain. Mediators like prostaglandins, bradykinins, substance P, histamine, cytokines, nitric oxide, which can be produced in damaged or injured tissue are also responsible for the modulation of nociceptive pathway\(^3\).

General strategy before the physician involves elimination of etiological factors involved in pathogenesis of the condition\(^12\). But treating the underlying condition may not immediately relieve pain and furthermore, some conditions like surgery and postoperative pain, burns, trauma and some stages of cancer are so painful that they necessitates the use of analgesic drugs. Painkillers are
the preferred drugs in those cases. Drugs which are frequently prescribed for the treatment of ache are Aspirin, Acetaminophen and other NSAIDs. Many of these drug act by similar mechanism\textsuperscript{12}. All these compounds are known to inhibit cyclooxygenase (COX) and all have anti-inflammatory actions in addition to their analgesic effect at appropriate doses. These drugs are useful in conditions of pain from different origin and hence they are generally consumed without the prescription by a physician. Amongst the COX inhibitors, drugs which interact with COX-1 are frequently given drugs. Chronic use of aspirin and other NSAIDS however results in side effects like gastric irritation which often results in reduction of the dose by physicians. Aspirin has an adverse effect related to the discomfort due to the damage of gastric mucosa, another side effect include gastric bleeding due to the effect of aspirin on platelets and blood hemostasis. The NSAIDs are less problematic as compared to the aspirin, but their use may result in nephrotoxicity in addition to the gastric irritation. Elevation of blood pressure has been observed in patients on NSAIDs which may be due to the retention of fluids. Acetaminophen another commonly used drug is associated with adverse effects on liver and gastric ulcers at higher doses \textsuperscript{13}.

There has been tremendous progress in understanding of the neurobiology of pain transmission, especially by virtue of application of modern techniques of electrophysiology and molecular biology\textsuperscript{3}. Another contributor for advancement in research for nociception is the need for new and more effective analgesics for clinical use. An alkaloid morphine is still being used despite of
well known undesirable side effect which is an indication of urgent requirement of analgesics that are free from adverse effects.

Despite of the abovementioned progress that has taken place in recent existence, there is a space for effective and potent analgesics, especially for chronic pain. Thousands of patients with intense and unrelenting pain, such as that resulting from cancer or injury, have to depend on drugs like morphine, despite its well-known side effects. We now need to realize that the development of analgesic drugs for the treatment of neurogenic and neuropathic pain is also urgently needed.

Many naturally occurring plants and their secondary metabolites have been reported to possess analgesic activities, however these studies are very preliminary in nature and very few of such study focus on the mechanism of action and the details of toxicity of such drugs. Researches on herbal medicines like morphine, capsaicin and cannabinoids have huge contribution in understanding of pathogenesis of pain and characterization of pain receptors along with endogenous ligands.

With the advent of knowledge and available database on possible targets and available strategies, new and effective analgesics can be obtained from various plants and secondary metabolites derived from these plants. As a result of which, the interest in the research involving higher plant-derived secondary metabolites as part of the search for new clinically useful drugs has tremendously increased not only amongst the researchers, but amongst the major pharmaceutical companies around the globe.
1.2 Inflammation

Human body or a part of any tissue when encountered with any injury, which may be caused by microorganism, trauma, chemicals, heat, or any other stimulus, various endogenous chemicals are released by the injured tissues which induce dramatic secondary changes in the surrounding uninjured tissues. This entire complex of tissue changes is called inflammation\textsuperscript{14}.

Inflammation is characterized by following events\textsuperscript{15},

1. Initial vasoconstriction followed by vasodilatation,

2. Altered permeability of vascular endothelium resulting in protrusion of plasma proteins and fluids into the interstitial space towards an injury,

3. Gathering of plasma fluid and proteins in the tissue results in the development of edema,

4. Clotting of this accumulated fluid in the interstitial space because of the fibrinogen,

5. Evacuation of leukocytes towards the site of an injury.

This entire complex of tissue changes is called inflammation. Mediators involved in this process are histamine, bradykinin, serotonin and prostaglandins and several different reaction products of the complement system along with the reaction products of the blood clotting system and multiple substances called lymphokines that are released by sensitized T cells. Apart from the destruction of the injurious agent and necrotized tissue, the inflammation frequently injures the surrounding living tissue cells\textsuperscript{14}. 
Though inflammation is a protective response induced by body tissues in response to various injuries or microbial invasion, it is a usually a hallmark of most disease processes and can cause discomfort which requires treatment and extended or chronic inflammation can lead to numerous diseases including rheumatoid arthritis, psoriasis and inflammatory bowel disease\textsuperscript{15}.

Current clinical approach to the treatment of various inflammatory diseases, consist of steroidal and nonsteroidal antiinflammatory drugs (Aspirin like compounds) exerting their pharmacological action by inhibition of prostaglandin synthesis catalyzed by the COX (Figure 1.1). COX-1 is a constitutive enzyme found in most tissues, including blood platelets. It has a regulatory role in the body, being involved in tissue homeostasis and is responsible for the production of prostaglandins involved in, for example, gastric cytoprotection, platelet aggregation, renal blood flow auto-regulation and the initiation of parturition. COX-2 isoenzyme in contrast to COX-1 is induced in inflammatory tissue and is responsible for the synthesis of prostonoid mediators of the inflammatory response\textsuperscript{16}. 

![Mechanisms of action of non-steroidal anti-inflammatory drugs](image)
**Figure 1.1: Mechanism of action of NSAIDs**

Drugs like Aspirin and the non steroidal antiinflammatory dugs have non-selective inhibitory action of the enzyme Cyclo-oxygenase (Figure- 1.1). COX-1 is fundamental iso-enzyme which is involved in the biosynthesis of prostaglandins\(^1\). These prostaglandins perform various roles like protection the gastric mucosa from ulceration, prevention of platelet aggregation via the prostaglandin derivative, thromboxaneA\(_2\) etc\(^1\).

Conventional NSAIDs are nonselective COX-inhibitors and affect both forms of isoenzyme, at varying degree. Most of the adverse effects of NSAIDs are related to their inhibition of COX-1, while their pharmacological antiinflammatory effect is a result of their inhibition of COX-2\(^1\). Compounds with a selective inhibitory action on COX-2 are now in clinical use, COX-2 selective inhibitors were developed to reduce the risk of gastrointestinal ulceration caused by non-selective NSAIDs. COX-2 selectivity reduces the risk of gastrointestinal adverse effects associated with classical NSAIDs. COX-2 inhibitors like rofecoxib and lumiracoxib are found to have reduced the incidence of upper gastrointestinal ulceration and bleeding by 50–60% or more compared to other NSAIDS\(^1\).

Aspirin and related NSAIDs are also known to affect renal function after their chronic use which may even lead to renal failure, particularly in older and other individuals with altered renal physiology. One must remember that prostaglandins are involved in maintenance of renal perfusion and function.
Unfortunately COX-2 selective inhibitors do not reduce the risk of renal impairment\textsuperscript{17}.

More importantly, COX-2 selective inhibitors, such as rofecoxib and celecoxib, which were introduced to decrease the gastrointestinal adverse effects associated with older non-steroidal anti-inflammatory drugs (NSAIDs) had to be withdrawn from market because of an increased risk of myocardial infarction and other thrombotic events\textsuperscript{18-20}. Because of these effects, safer compounds are needed and therefore, alternate remedies like plant remedies have become increasingly popular and are often preferred to synthetically derived pharmaceuticals.

Inflammation or inflammatory disorders have also been treated with a number of medicinal plants from centuries\textsuperscript{21}. Plants exerting antiinflammatory effects have been studied and it is believed that certain secondary metabolites interfere with the cascade of the inflammatory reactions and hence presence of such phytoconstituents is responsible for their pharmacological effects. Pharmacological evidence for the anti-inflammatory activity of some of these medicinal plants has been established according to the findings of research, many of such phytoconstituents are known to inhibit the release of pro-inflammatory mediators, migration of leukocytes under inflammatory stimulus with consequent release of the cytoplasmic contents at inflammation sites, activation of complement sequence, etc\textsuperscript{21}.

1.3 Rheumatoid arthritis
Rheumatoid arthritis (RA) is a systemic, chronic destructive inflammatory disease affecting various tissues but principally the joints to produce a nonsuppurative proliferative synovitis. The progression of the disease involves destruction of cushion inside the bone resulting in clinical disability.

Arthritis is most common clinical condition involving chronic inflammation in developed countries and rheumatoid arthritis remains the common cause of disability with one of every three patients likely to become severely disabled. The prevalence of RA is approximately 1% of total population and hence it is very common condition with three to five times more prevalence in females than their counterparts. Occurrence of the disease is more common in second to fourth decade of life.

Rheumatoid arthritis causes a broad spectrum of morphologic alterations; the most severe occur in the joints. RA is an example of symmetric arthritis, affecting the petite joints of limbs. The major histological changes include chronic synovitis which is characterized by following:

- Hyperplasia and proliferation of synovial cell;
- Infiltration of chronic inflammatory cells in the synovium;
- built-up of network of new blood vessels;
- Aggregation of plasma proteins like fibrin with neutrophils in the joint;
- Synovial degeneration with bone erosion and granuloma formation.

Drug therapy of RA includes Disease modifying anti-rheumatoid drugs (DMARDs) and the NSAIDs. DMARDs are known to inhibit and may reverse the pathological changes of the disease whereas the NSAIDS are known to cause
symptomatic relief. Other class of compounds like glucocorticoids and immunosuppressant are also used for the management of the disease\textsuperscript{23}. This clinical condition has been treated with antiinflammatory drugs and disease-modifying anti-rheumatic drugs (DMARDs) which in most cases, have been proved to be of only limited value\textsuperscript{24}.

According to the present clinical opinion, most of the above mentioned drugs are known to control of pain and the inflammation associated with joint synovitis, but their claim to inhibit or cure the disease is optimistic there is little if any effect on pathological condition of the disease. The pharmacotherapy of arthritis with current allopathic drugs is known to have various limitations and long term treatment with such agent results in profound side effects and toxicity and the drugs which are relatively safer are not cost friendly and still have a risk of hypersensitivity\textsuperscript{24}.

Herbal drugs on the other hand have received a consistent interest not only from people with RA but the researchers as well. Herbal drugs are being widely prescribed for arthritic disease around the world and the demand of herbal drugs has only increased in recent existence. Therefore the research on phytomedicines has become an important goal for finding the drugs that can control the destruction of joints in arthritis and offer safe, tolerable and economic alternative to the society\textsuperscript{24\& 25}.

1.4 Reactive oxygen species

Reactive oxygen species (ROS) are a group of highly hasty species which are routinely produced inside our body and perform some physiological tasks
in the body\textsuperscript{26}. It includes a role of signal transduction in various inflammatory reactions like vasodilation, neutrophil emigration and phagocytosis. Excessive generation of such ROS however is associated with deterioration of some biomolecules inside the body\textsuperscript{26}.

Oxidation of LDL gives rise to the intermediates which do not bind to the LDL receptors, but instead induce macrophage reaction which result in formation of a foam cell and can lead to the formation of atherosclerotic plaques. ROS can also activate various damaging pathways of progression of various disorders like diabetes, neurological disorders and cardiovascular disorders\textsuperscript{9}.

Exogenous sources of ROS which are generated from external environment are summarized in figure 1.2 Radiolysis can take place in human body when a person is exposed to ionizing radiation\textsuperscript{26}.

Radiolysis begins with a loss of an electron by water to become highly reactive intermediate. This is followed by a three-step chain reaction in which, consequent reactive radicals are produced, firstly $\text{H}_2\text{O}$ is transformed to $-\text{OH}^-$, then to $-\text{H}_2\text{O}_2^-$, $-\text{O}^-$ and finally to $-\text{O}_2^-$. $-\text{OH}^-$ is most active and takes out an electron from the molecules coming across its journey and thereby sustaining the cascade of generating new free radicals. Though $-\text{OH}^-$ is most reactive, the majority of the damage is done by $-\text{H}_2\text{O}_2^-$ by virtue of its slow reaction which provides sufficient time period for deep penetration in to the biomolecules\textsuperscript{26}.

Inside body, radicals are produced in different tissues at various sites like cell membranes, mitochondria, peroximes and endoplasmic reticulum. Production of ATP,
through the reduction of oxygen to water in the mitochondria occurs through the donation of 4 electrons to oxygen to produce water. During this process several ROS leak from the mitochondria into the intracellular environment and hence mitochondrion serves as the major endogenous source for ROS production. Production of mitochondrial ROS is found to be increased in the aging cell. Another major source for ROS is enzymes, most enzymes produce ROS as a by-product of their activity, e.g. formation of superoxide radicals by xanthine oxidase, nitric oxide synthase that yields NO radicals. The pathogenic conditions involving interaction of the White blood cells with infectious micro-organism and several other injuries also contribute significantly to the production of ROS. Various diseased states are similarly responsible for the increased production of ROS\textsuperscript{11}.

1.5 Antioxidants

The free radicals that are generated in the body require an electron from neighboring molecule for their neutralization, a process called as oxidation, which results in production of another free radical in the proximity\textsuperscript{11}. This newly formed free radical again search for another molecule for neutralization, giving rise to a cascade of reactions that can damage to several other molecules\textsuperscript{12}.

Antioxidants are the substances or compounds that stop this chain of reaction and neutralize harmful free radicals that can damage living cells, spoil food and other industrial products\textsuperscript{12}.

Various sources of antioxidants include proteins synthesized in the body along with some dietary supplementary consisting micronutrients. For
industrial purposes, some synthetic antioxidants are routinely added to the reactions\textsuperscript{12}. 

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{sources_of_oxygen_species.png}
\caption{Sources of oxygen species}
\end{figure}

Antioxidant research has attracted many researchers who were inspired by a thought that many degenerative human diseases like cancer, diabetics, cardio and cerebro-vascular diseases involves damage due to oxidative stress and involves degeneration of lipids, proteins and nucleic acids by free radicals\textsuperscript{11}. With the possibility of the toxic effects, chemical antioxidant substances have been facing a criticism and as a result of which recent studies are investigating the medicinal plants for their antioxidant and free radical scavenging potential. Various secondary metabolites present in plants viz.
phenolic compounds, flavonoids, tannins have been reported to possess strong antioxidant activities\textsuperscript{11}.

1.6 Helminthes infections and anthelmintic drugs

Helminthes infections are common of all chronic infections and it is estimated that half of the population harbor worms in the gastrointestinal tract; children may be infected with one or more species of helminthes and many remains infected throughout their life span. Worms like threadworm do not cause only moderate discomfort confined to the GIT, but some species like schistosomiasis and hook worm are known to cause serious infections\textsuperscript{12}.

Drugs that are prescribed for infections caused by worms produce their action by altering the motility of the worms there by predisposing them to immune attack by host or by elimination outside the body\textsuperscript{23}. Differences of the nature of metabolic processes of these parasites results in selectively toxic effect of anthelmintic agents but as there are intra species variation, spectrum of activity of any drug becomes a constrain. Looking at the prevalence of helminthes infections, treatment of helminthiasis is one of very great practical therapeutic importance\textsuperscript{12}. There is an urgent need for alternative therapies or drugs for cure of helminthes infections\textsuperscript{12}.

1.7 Cuscuta reflexa
*Cuscuta reflexa* Roxb. belonging to family Convolvulaceae, (Dodder) is well known as *Amarwel* in Ayurveda\(^9\). It is a parasitic climber found commonly throughout India. One of the important characteristic of this climber is it do not have leaves neither have roots underground and grows as a twinner on other plants and hence called as ‘Akaswel’. Due to its recurrence on host plant every season it is also referred as *Amarwel* (Immortal twinner)\(^9\).

Right from the down line of history, *Cuscuta reflexa* has been used for various purposes viz. as a purgative, in the treatment of liver disorders, cough and itching and for its carminative and anthelmintic properties\(^10\). The parasite is reported to possess preliminary antiinflammatory activity\(^27, 28\). Present study is an attempt to explore detail antiinflammatory activity of extracts of *Cuscuta reflexa* using different models of acute as well as chronic inflammation. The emphasis was also given to find out mechanism involved in behind the antiinflammatory effect.

Plants rich in flavonoids which are known antioxidants can have protective effect against a variety of diseases. The protective effects of flavonoids in biological systems are by virtue of their capacity to transfer free radical electrons and chelate metal catalysts\(^29\).

*Cuscuta reflexa* has been used for various purposes viz. as a purgative, in the treatment of liver disorders, cough and itching and for its carminative and anthelmintic actions\(^9\). The parasite is reported to possess hypotensive, hypoglycemic, antiviral, cytotoxic and preliminary antiinflammatory activity.
Extracts of the reported the plant are also reported to exert spasmolytic on small intestine of guinea pig and rabbit, have antimicrobial activity\textsuperscript{30-33}.

Although naturally occurring secondary metabolites derived from this plant with some pharmacological properties are found in the literature\textsuperscript{30}, the great majority of these reported studies are preliminary in nature and hence it has become clear from this review that there is a need of extensive research on the pharmacological properties of the plant which may be expected to have therapeutic benefit in the management of different disorders.

Looking at the increasing need and demand of better and safer drugs with lot of interest in herbal medicines and the knowledge gap between the several ethnomedical claims and the systematic investigations on the plant \textit{Cuscuta reflexa}, it was decided to explore the biochemical, pharmacological and toxicological investigations of extracts of this plant using animal models.