### CHAPTER-2

**LITERATURE REVIEW**

**TABLE OF CONTENTS**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of the sub-title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Literature review</td>
<td>23</td>
</tr>
<tr>
<td>2.1</td>
<td><em>Cuscuta reflexa</em></td>
<td>23</td>
</tr>
<tr>
<td>2.2</td>
<td>Pain</td>
<td>30</td>
</tr>
<tr>
<td>2.3</td>
<td>Inflammation</td>
<td>34</td>
</tr>
<tr>
<td>2.4</td>
<td>Nonsteroidal anti-inflammatory drugs</td>
<td>36</td>
</tr>
<tr>
<td>2.5</td>
<td>Adverse effects of NSAID’s</td>
<td>38</td>
</tr>
<tr>
<td>2.6</td>
<td>Plants as a source of anti-inflammatory drugs</td>
<td>39</td>
</tr>
<tr>
<td>2.7</td>
<td>Liver toxicity and hepatoprotective drugs</td>
<td>40</td>
</tr>
</tbody>
</table>
2. LITERATURE REVIEW

2.1 Cuscuta reflexa (Roxb.)

2.1.1 Introduction

Cuscuta is a species of leafless yellow or reddish, twinning, parasitic annuals, the characteristic of the species being absence of chlorophyll and leafless stems which are slender and sometimes filiform. The flowers are small, white or rose colored found in sessile and pedicellate form. The important phytoconstituents found in the species are, Cuscutin, Cusculatin and amarbelin.

Following species are found to be useful medicinally, C. epithymum linn., C. europoea linn., C. lupuliformis Krocker, C. chinesis Lam., C. japonica Chois., C. racemosa Marc., C. umbellaat Humb. and C reflexa Roxb\textsuperscript{34, 35}.

Convolvulaceae is a family of about 55 genera and 1650 species. Most are annual and perennial herbs often with twining stems. The genus Cuscuta consists of parasites which are sometimes placed in a separate family, the Cuscutaceae. Species of dodder are parasitic on clover and on flax. Plants from Cuscuta species are stem parasites, found world-wide. Minimum 150 different species are available, having similar morphological properties. The taxonomic characters of the genus are limited almost entirely to the flower, fruit and inflorescence, as the vegetative parts of the plants are very simple in structure and show great uniformity, in most of the cases, mature flowers are essential for correct identification of the respective species\textsuperscript{36}.

2.1.2 Botanical description
Cuscuta reflexa Roxberg i.e. dodder belonging to the family Convolvulaceae is a wild growing hair like golden colored stem parasite which is called as Amarwel because of its immortal occurrence in every season on some host species. The plant is also referred as Akashwel in Hindi because it covers the whole host plant and keeps growing in the direction of sky. Cuscuta reflexa is characterized by golden yellow to yellowish green stems which are slender, along with close twining, branching with red or brown spots having a diameter of two to three millimeter. Flowers are solitary to multiple present laterally on stems present in a culture with branches; pedicles are usually curved, short and glabrous. Bracts are 1.5 mm long, ovate, oblong and fleshy. Calyx is divided at the base with lobes of 3mm. The other morphological characteristics include, presence of Pedicel along with brown colored peduncle, five sepals, ovate with tubercles abaxially, white to cream colored Corolla, with deciduous lobes. Seeds 1-4, dark brown, oblong, 4 mm.

C. reflexa found in the Indian subcontinent is used in a number of folk remedies; an alcoholic extract of the plant has hypotensive and bradycardiac effects, the plant has been reported to parasitize Cuminum Cyminum but it does not grow on neem, it has also been successfully grown in vitro in a liquid medium without a host. The herb has bitter sharp taste; expectorant, carminative, tonic, purgative diaphoretic properties. The sows have a vinegary taste; and claimed to be used as hepatoprotective, antipyretic, sedative analgesic, and emmenagouge. Plant is useful in the form of decoction and powder for the purification of body. Another formulation is administered in eye
cavity. Its decoction also causes purgation whereas the cold concentrate is taken for detoxification\textsuperscript{36}. The seeds are regarded as carminative and for this purpose are boiled and placed over the stomach. They are also applied as an anodyne. Native doctors of Sindh and Punjab regard the seed of this plant as alterative and used to purify the blood. Stems are specially used in bilious disease\textsuperscript{35}. 
Figure 2.2: *Cuscuta reflexa* Roxb.
<table>
<thead>
<tr>
<th><strong>Kingdom</strong></th>
<th><strong>Plantae – Plants</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subkingdom</strong></td>
<td><strong>Tracheobionta – Vascular plants</strong></td>
</tr>
<tr>
<td><strong>Superdivision</strong></td>
<td><strong>Spermatophyta – Seed plants</strong></td>
</tr>
<tr>
<td><strong>Division</strong></td>
<td><strong>Magnoliophyta – Flowering plants</strong></td>
</tr>
<tr>
<td><strong>Class</strong></td>
<td><strong>Magnoliopsida – Dicotyledons</strong></td>
</tr>
<tr>
<td><strong>Subclass</strong></td>
<td><strong>Asteridae</strong></td>
</tr>
<tr>
<td><strong>Order</strong></td>
<td><strong>Solanales</strong></td>
</tr>
<tr>
<td><strong>Family</strong></td>
<td><strong>Cuscutaceae/ Convolvulaceae</strong></td>
</tr>
<tr>
<td><strong>Genus</strong></td>
<td><strong>Cuscuta L. – dodder</strong></td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td><strong>Cuscuta reflexa Roxb.</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Language</strong></th>
<th><strong>Synonym</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabic</td>
<td>Kashus, Sharulzabia</td>
</tr>
<tr>
<td>Assam</td>
<td>Amarlati</td>
</tr>
<tr>
<td>Bengal</td>
<td>Algusi, Hadialgusilutta</td>
</tr>
<tr>
<td>English</td>
<td>Dodder</td>
</tr>
<tr>
<td>French</td>
<td>Cuscute</td>
</tr>
<tr>
<td>Gujarati</td>
<td>Amarbel</td>
</tr>
<tr>
<td>Hindi</td>
<td>Akashbel</td>
</tr>
<tr>
<td>Marathi</td>
<td>Amarvela</td>
</tr>
</tbody>
</table>
2.1.3 Chemical constituents and uses

*Cuscuta reflexa* is known to contain various secondary metabolites like flavonoids, glycosides, alkaloids, cuscutin, cuscutalin and amarvelin. The fresh plant yields scoparone, quercetin and hyperoside and phenolic compounds. The main active principles of the plant are cusculatin and cuscutin. β-sitesterol, stigmasterol, kaempferol, dulcitol, myricetin, quercetin have also been isolated from the *C. reflexa*. Lupeol is a pharmacologically active triterpenoid which has been extracted. Cuscuta is also known to contain antibacterial and antiviral substances\(^{35, 38}\).

*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. 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\(^{39-42}\).
Although naturally occurring secondary metabolites derived from this plant with some pharmacological properties are found in the literature\textsuperscript{39}, 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. Hence, it was decided to explore the biochemical, pharmacological and toxicological investigations of extracts of this plant using animal models.

It has become clear from the literature review that the plant contains several phytoconstituents like phenolics and flavonoids and has been used for the treatments of various ailments from ancient times. The literature also includes several claims of antiinflammatory, analgesic, antioxidant and hepatoprotective activity of the plant. However there is a need of scientific validation of these claims by the use of systematic approach using experimental animals.

By virtue of the secondary metabolites, the plant may possess antioxidant activity and looking at the role of oxidative stress in various diseases, it was expected that the plant extract may exhibit protective and curative effect in various condition involving pain, inflammation and liver diseases.

2.2 Pain

The word Pain is derived from a Greek word poine, which means penalty or punishment\textsuperscript{19}. Precise definition of pain is difficult to find as it is a subjective feeling. It is a reaction to any noxious stimuli causing tissue injury and inflammation. Pain is the fundamental symptom of many diseases and
hence becomes one of the important concern for health care society\textsuperscript{19}. Pain is as oldest medical problem since civilization and peoples have always been trying to get rid of it. Even today, the burden of pain can be understood from the epidemiology of pain, hundreds of millions civilians are facing the troubles of pain around the globe\textsuperscript{43}. Unfortunately, pain often remains undertreated and continues to contribute to the morbidity in society\textsuperscript{43}.

Two distinct categories of pain can be identified according to the origin of stimulus i.e. the one arising from the viscera or organs thereof is called as visceral pain and one arising from external organs is regarded as somatic pain\textsuperscript{44}. The pathogenesis of pain involves an activation or sensitization of nociceptors which are small-diameter $\delta$-fibers the activation of whose causes sensation of pain\textsuperscript{43}. These fibers have sensory terminals in peripheral tissues and are stimulated by various mechanical, chemical and thermal stimulus\textsuperscript{43}. Processing of nerve impulses generated after activation of nociceptors involves peripheral and central mechanisms that take the impulses to the CNS. In case of acute condition, this activation is short-lived, but in some situations, this may give persistent and chronic pain\textsuperscript{43, 44}.

Primary afferent fibers differ from other receptors by their higher threshold, because they are not activated unless and until the stimulus is crossing the threshold value and hence only moderate to extreme stimulus activates them\textsuperscript{23}. Many of these fibres are non-myelinated C fibres; this group is known as C-polymodal fibers. The $A\delta$ fibers are known for their rapid
propogation rate giving rise to rapid firings. Stimulus from periphery is known to activate them.

Internal organs and their tissues are also innervated by such fibres, activation of which is associated with the noxious changes inside these organs, however these fibres are set on high threshold values and hence requires potentially strong stimulus for their activation. Skin which is the largest organ is supplied with the C-fibres which are not mylinated and are activated upon various stimuli23.

Acute pain is usually associated with a signal of an injury or of diseased condition involving43.

**Stimulation**- Noxious stimulus sensitizes and/or stimulates nociceptors and causes the release of neural chemicals that cause activation of these receptors. This activation leads to the production of an action potential43.

**Transmission**- The action potential continues from the site of noxious stimulus to the spine followed by further areas on CNS43. Transmission takes place in at least five pathways:

- Spinal-thalamic pathway
- Spinal-reticular pathway
- Spinal-mesencephalic pathway
- Dorsal column postsynaptic spinomedullary pathway
- Propriospinal multisynaptic ascending systems

**Perception**- Includes conscious experience of pain.
**Modulation** - this consists of inhibition of nociceptive impulses. Certain analgesic peptides which are released by these fibers are known to inhibit the activation and propagation of the impulses towards the CNS\(^{43}\).

But as the severity of pain increases, the intensity and severity of pain also increase and if left unnoticed, it can give rise to various complications like ischemia, bradycardia, elevated blood pressure and altered state of emotion. Usually, acute pain subsides quickly as the healing process decreases the pain-producing stimuli; however, chronic pain persists for a longer periods with some additional complications than seen with acute pain\(^{43}\). Treatment of painful conditions involves use of various agents called as analgesics. Opium alkaloids and their analogues are known to exert severe adverse effects and hence their application is limited; aspirin and related nonsteroidal anti-inflammatory agents are accepted on the other hand for the therapy of such conditions\(^{23}\). Many of such drugs are found to inhibit biosynthesis of prostaglandins produced in painful conditions and there by an effect on rate of firing and propagations of nociceptors. However, these drugs are also associated with several adverse effects, like gastrointestinal irritation, bleeding, accumulation of fluids etc\(^{23}\). Many of the patients with severe pain are given opiate analgesics despite of their adverse effects\(^{23}\).

Plant derived substances have contributed heavily in the field of drug discovery, looking at the urgent need in the field of analgesics; researchers have shifted their focus to naturally occurring substances\(^{4}\). Various secondary metabolites present in medicinal plants have been found to possess analgesic
effect and can therefore be developed for the management of painful condition; however extensive research on these plants should be done to support the possible opportunities for development of newer and safer analgesics.

2.3 Inflammation

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent and is an effort to destroy the etiological agent and thereby protect the host. The agents causing inflammation may be as under:

1. Infective agents like bacteria, viruses and their toxins, fungi, parasites.
2. Immunological agents like cell-mediated and antigen-antibody reactions.
3. Physical agents like heat, cold, radiation, mechanical trauma.
4. Chemical agents like organic and inorganic poisons.
5. Inert materials such as foreign bodies.

Thus, inflammation is distinct from infection—while inflammation is a protective response by the body to variety of etiologic agents (infectious or non-infectious), while infection is invasion into the body by harmful microbes and their resultant ill-effects by toxins. Inflammation involves 2 basic processes with some overlapping, viz. early inflammatory response and later followed by healing. Though both these processes generally have protective role against injurious agents, inflammation and healing may cause considerable harm to the body as well e.g. anaphylaxis to bites by insects or reptiles, drugs, toxins, atherosclerosis, chronic rheumatoid arthritis, fibrous bands and adhesions in intestinal obstruction.
The Roman writer Celsus in 1st century A.D. named the famous 4 *cardinal signs of inflammation* as; *rubor* (redness); *tumor* (swelling); *calor* (heat); and *dolor* (pain). To these, fifth sign *functio laesa* (loss of function) was later added by Virchow. The word inflammation means burning. This nomenclature had its origin in old times but now we know that burning is only one of the signs of inflammation. Inflammatory response can generally be classified in two classes

Acute inflammation; and chronic inflammation.

In inflammatory response which persists from few hours till few days (15 days) is generally regarded as acute inflammation. Whenever the injurious agent persists even after acute inflammation, chronic inflammation generally takes place.

Important characteristics of acute inflammation are

1. Edema formation which is generally due to accumulation of contents of blood vessel i.e. plasma and fluid;
2. Activation of platelets followed by release of mediators;
3. Migration of neutrophils towards the place of inflammation;
4. Phagocytosis.

Chronic inflammation on the other hand exists for longer time and is characterized by involvement of lymphocytes, macrophages, plasma cells and granulomatous inflammation.

2.4 Nonsteroidal Anti-Inflammatory Drugs (NSAIDS)
Though Inflammation is a protective response, it is now understood that poor control of inflammation in the body is responsible for the damage of surrounding tissues and organs. Many chronic inflammatory conditions are an example of such uncontrolled physiological response. Many chemical mediators released during inflammation are responsible for such degradation, requiring the treatment by anti-inflammatory agents.

NSAID are a group of heterogeneous compounds which possess analgesic, antiinflammatory and antipyretic actions. These drugs in contrast to opiates do not produce dependence and do not produce respiratory depression. They are also called as non-narcotic, non-opiate and Aspirin like analgesics.

These drugs affect the biosynthesis of ecosanoids and thereby exert analgesic, antiinflammatory effect; they also inhibit the cytokines released from inflammatory tissue. The enzyme responsible for the production of prostaglandins is prostaglandin synthase also known as cyclo-oxygenase (COX). It is this enzyme which transforms arachidonic acid to PGG₂ and PGH₂ and subsequently to thromboxane and various other prostaglandins. Two isoforms of the enzyme COX are found in human body, COX-1 and COX-2. COX-1 is basically inherent isoform synthesized in many tissues, whereas inflammation and related tissue changes stimulate COX-2 production. Importantly, COX-1 produced in gastric mucosa is responsible for protection of gastric epithelium from the harmful effects of pepsin and hydrochloric acid. Inhibition of COX-1 synthesis by conventional antiinflammatory drugs is responsible for the adverse effects related to the gastric mucosa and hence it is
believed that selective action on COX-2 would be beneficial in the treatment of inflammatory conditions. These drugs also possess analgesic activity, especially, inflammation associated sensitization of nociceptors\textsuperscript{23}.

2.5 Adverse effects of NSAID therapy

There are varieties of adverse effects of NSAIDs therapy\textsuperscript{47}, some of potential adverse effects are as follows

**Gastrointestinal**

These are the most frequent adverse effects associated with these drugs and include ulceration, nonselective inhibition of COX, and resultant inhibition of biosynthesis of cytoprotective prostaglandins results in various adverse effects ranging from gastric discomfort, vomiting, to ulceration and severe gastritis depending upon the individual agent and dose consumed. These adverse effects are common and seen in one-third of the populations consuming these drugs.

Ulceration which is byfar most severe adverse effect is associated with local small lesions of erosion on the principal layer of the wall of GIT. There may be single or multiple ulcers and ulceration is usually associated with the anemia and blood loss which may be lethal in extreme cases where concominant consumption of alcohol may worsen the situation. Other risk factors for mucosal injury, includes *H. pylori* infection and concurrent use of glucocorticoids. Drugs having preferential or direct action on COX-2 are showing decreased incidences of adverse effects and this has provided the impetus to the researchers for the research on COX-2 preferential drugs\textsuperscript{47}.
**Cardiovascular**

Incidences of severe and life threatening thrombo-embolism and MI have been associated with the drugs like rofecoxib and celecoxib which had selective action on COX-2. NSAIDS are also associated with accumulation of Sodium-chloride and H₂O in hypertensive individuals, diminishing the therapeutic effect of anti-hypertensives⁴⁷. COX-2 inhibitors are frequently associated with cardiological adverse effects as compared to other antiinflammatory drugs⁴⁷.

**Analgesic Nephropathy**

Incidences of acute and chronic renal failures have been reported with the chronic use of antiinflammatory drugs⁴⁷.

Apart from various adverse effects, Aspirin like compounds have several other disadvantages like toxicity, recurrence of symptoms after discontinuation of the treatment, economic burden etc., therefore the need of an hour is the search for alternative therapy which would be safe, effective and economic.

**2.6 Plants as a source for anti-inflammatory agents**

Herbal medicines have played a vital role in the process of drug discovery³. Many medicinal plants like Willow bark, by virtue of their active secondary metabolites, have served as a lead for drug design and discovery. Plants contain thousands of secondary metabolites and an effect produced by a plant may be a collective effect due to the synergy of more than one compound. Many medicinal plants have been used from thousands of years for the treatment of inflammatory conditions and many classes of secondary metabolites i.e. phenolics, flavonoids, terpenoids, alkaloids have been found to possess potent
anti-inflammatory activity. The use of herbal medicines is becoming more and more popular. Physicians around the globe prefer to prescribe herbal medicines over allopathic drugs.

Though many plant species have been explored for biological activities, pure compounds responsible for their activities have not been explored and hence there is a need for isolation and systematic investigation of pure compounds\(^2\).

Our part of the world has a huge heritage of medicinal plants and hence can prove to be a source of various medicinal plants which can contribute to the healthcare system by providing the drug molecules or intermediates required for the production of various drugs\(^2\).

2.7 Liver toxicity and hepatoprotective drugs

Liver is an important organ involved in the biotransformation and metabolism of not only nutrients and food, but also drugs and other xenobiotic substances. Liver plays central role in the process of detoxification and elimination of chemicals and metabolites generated in-vivo. Many of the endogenous substances and some of the drug metabolites along with infectitious agents and habit forming agents are known to cause damage of the hepatocytes resulting in decreased functional capacity of the liver. Liver is equipped to tolerate these insults with its inherent protective mechanisms, but over exposure to the insults results in hepatic injury\(^4\).

Such chemicals that cause liver injury are called hepatotoxins. Many inorganic as well as organic chemicals are known to cause toxicity of the
hepatocytes; for an example the metals like copper, iron, or toxins and mycotoxins are also known to cause potential hepatotoxicity\textsuperscript{50}. Some of the environmental elements also exert the threat of hepatotoxicity, in addition, some peoples are exposed to the occupational pollutants due to their occupation\textsuperscript{50}.

Hepatotoxicity has been associated with a variety of changes that are accompanied like deterioration of cellular framework, increased levels of MDA which is an intermediate of lipid peroxidation, and reduced levels of livers inherent anti-oxidant enzymes. Secondary changes may include hypercholesterolemia and alteration of various biomarkers\textsuperscript{49}. Despite of the development in the field of medicine and biotechnology, very few synthetic drugs are available for the treatment of hepatic disorders. However many medicinal plants are claimed to be hepatoprotective by virtue of the presence of antioxidant substances like, phenolic compounds, flavonoids and other such free radical scavangers\textsuperscript{49, 50}. The phytochemical information of the parasitic plant \textit{Cuscuta reflexa} reviewed in this section and the critical issues in the clinical areas addressed, following section will propose a theoretical analysis for pharmacological, biochemical and toxicological investigations on the plant \textit{Cuscuta reflexa}. 