Summary, Conclusion and recommendations:

Present investigation highlights biochemical, pharmacological and toxicological investigations on the plant *Cuscuta reflexa* in animal models of pain, inflammation, hepatotoxicity in laboratory animals.

The study also includes toxicological investigations using acute and sub-acute toxicity study on mice and rats respectively. *In vitro* assays for antioxidant and cytotoxicity properties have also been conducted. Finally activity of the extracts against helminthes infection has been investigated.

This study suggests that pathology of pain, inflammation and liver disease involves active role free radicals generated by various pathological changes associated with the disease.

Pain is an inevitable component of many diseases and hence it is of prime concern. The oldest analgesic is opiate and is still being used for some critical painful conditions, despite of well known side effects. Herbal medicines have provided many useful drug discoveries, the hope is from the herbal medicines.

Inflammation is the protective response exerted by our body against variety of injurious stimuli; however poor control over this immune response is frequently associated with the damage to the surrounding tissue. Many of the diseases involve inflammation of tissues. The current therapy of the inflammation constitutes of steroidal and nonsteroidal antiinflammatory drugs; however these drugs are associated with severe adverse effects including gastric ulceration, renal failure, hepatotoxicity etc. Hence there is a need of development of newer and safer drugs.
It is the imbalance between the oxidative stress and protective mechanisms exerted by the body, which causes the changes in the body which predisposes to the liver injury.

Herbal medicines are generally preferred over the allopathic medicines because of a general belief that these drugs are cheaper, readily available and more importantly safer as compared to other drugs. Which is why, more than 75% of the population prefers to use herbal medicines.

*Cuscuta reflexa* is a parasitic climber found all over India. It is a leafless unique plant well known for some of its characteristics like to surround and cover host plant and its immortal nature, i.e. to be able to grow every season on the same plant, perhaps which is why called as Amarwel i.e. the immortal twinner. The plant has been claimed to have analgesic, antiinflammatory and hepatoprotective properties and have been used by ancient peoples in the form of crude powder, decoction for various ailments. Despite of the advancement of the herbal medicines there is little, if any information is available on the safety profile and efficacy of this plant. Though some studies have been conducted, they are preliminary in nature and hence to validate the claims of analgesic, antiinflammatory, antioxidant, hepatoprotective and anthelmintic properties, this study was aimed to use various animal models to investigate biochemical, pharmacological and toxicological properties of the extracts of *Cuscuta reflexa*.

Acute toxicity study was performed on various extracts of *Cuscuta reflexa*, and the result indicated that the LD₅₀ is more than 2000 mg/kg. Repeated dose sub-acute toxicity study indicated that the extracts of *Cuscuta reflexa* are
devoid of any remarkable toxic effects on liver, heart, kidneys, and haemopoietic system.

Models used for evaluation of analgesic activity were hot plate test, acetic acid induced writhings and formalin test. The latency of the animals treated with *Cuscuta reflexa* was significantly increased as compared to the control animals. Writhings produced by intraperitoneal injection of acetic acid was significantly inhibited (up to 74%; where Inhibition by DCS is 84%) as compared to the control animals. Similarly the formalin induced licking was significantly (up to 67%; where Inhibition by DCS is 87%) inhibited in the animals treated with *Cuscuta reflexa*. Taken together the results of these three tests indicate a significant analgesic effect of extracts involving a complex mechanism associated with the inhibition of mediators of pain and stabilization of the nociceptors.

Antiinflammatory activity was investigated using various models for different phases of inflammation. Carrageenan induced paw edema along with mediators induced paw edema was used for induction of acute inflammation.

Subplantar injection of a carrageenan solution causes acute paw edema in control animals which is significantly (up to 64%; where Inhibition by DCS is 69%) inhibited by the extracts of *Cuscuta reflexa*. Similarly mediators of inflammation also cause paw edema and significant inhibition of the mediator induced paw edema by *Cuscuta reflexa* indicates inhibition of the action of important mediators of inflammation.
Sub-chronic inflammation caused by surgical implantation of cotton pellets was significantly (up to 49%; where Inhibition by DCS is 69%) inhibited by various extracts of *Cuscuta reflexa*. Minimal gastric ulceration in the animals treated with MECR and AECR gave an indication of good gastric tolerability and inhibition of acetic acid induced vascular permeability suggests that the antiinflammatory effect exerted by various extracts of *Cuscuta reflexa* may involve the inhibition of mediators of inflammation associated with the vascular events of inflammation; similarly inhibition of leukocyte migration and red blood cell stabilization against hypotonicity suggest complex mechanism involved in the antiinflammatory effect of *Cuscuta reflexa*. 

Methanolic and aqueous extracts of *Cuscuta reflexa* were evaluated for anti-arthritis activity using conventional animal models of arthritis like formalin induced arthritis and Freund’s complete adjuvant induced arthritis. Both MECR and AECR show promising anti-arthritis activity in both models of arthritis. Various biochemical, hematological and histological changes associated with FCA induced arthritis which include reduced body weight, enlargement of spleen, thymus; elevation in serum markers like ALT, AST, ALP, total bilirubin, total cholesterol, triglycerides, lipid peroxidation and reduction of serum protein levels were normalized by the chronic treatment with MECR and AECR giving an indication of usefulness of *Cuscuta reflexa* in the treatment of arthritis. Along with other probable mechanisms involved in anti-inflammatory effect, inhibition of proinflammatory cytokines TNF-α and IL-2 by
treatment with MECR and AECR is responsible for its protective effect in this chronic inflammation.

Antioxidant and hepatoprotective potential of *Cuscuta reflexa* was investigated using *invitro* tests of free radical scavenging assay and CCl₄ induced hepatotoxicity. Extracts of *Cuscuta reflexa* possess significant free radical scavenging activity and also exert potent hepatoprotective activity. Biochemical and histological alterations associated with CCl₄ are successfully normalized by the treatment with methanolic extract of *Cuscuta reflexa* and this may be attributed to the antioxidant activity exerted by extracts of *Cuscuta reflexa*. Presence of phenolics and flavonoids could be responsible for this effect.

In an another study various extracts of *Cuscuta reflexa* exerted significant anthelmintic activity and this could be due to the presence of tannins.

Thus in conclusion, biochemical, pharmacological and toxicological investigations of *Cuscuta reflexa* on experimental models of pain, inflammation and hepatotoxicity revealed that extracts of *Cuscuta reflexa* exhibit dose dependent analgesic, antiinflammatory and hepatoprotective activity which may be due to the antioxidant phytoconstituents.

Chronic administrations of do not produce any sign of toxicity; giving an indication that it is safe.

Antiinflammatory response produced by extracts of involve complex mechanism including inhibition of inflammatory mediators histamine and serotonin; inhibition of prostaglandin synthesis, inhibition of leukocyte
migration, stabilization of lysosomal membrane, inhibition of pro-inflammatory cytokines.

Oxidative damage associated with chronic inflammation is normalized by chronic administration of extracts of *Cuscuta reflexa*. This effect is due to the antioxidant activity of *Cuscuta reflexa*.

Hepatotoxicity induced by CCl₄ is significantly inhibited by chronic administration of extracts of *Cuscuta reflexa*. The biochemical analysis revealed normalization of all changes associated with liver damage and the histological analysis confirmed the same. Hepatoprotective property of *Cuscuta reflexa* may be attributed to its free radical scavenging ability along with stimulation of biosynthesis of antioxidant enzymes.

The potent analgesic, anti-inflammatory, antioxidant and hepatoprotective activity exerted by extracts may be attributed to the presence of phenolics and flavonoids and one of the fractions characterized to be quercetin.

Extracts of *Cuscuta reflexa* also exhibited potent and dose dependent cytotoxic and anthelmintic activity which may be attributed to the presence of tannins.

We recommend that the plant holds a strong potential and should be explored for various other pharmacological properties, specially the diseases involving the pathological role of oxidative stress.

The present study using preliminary animals experimentation demonstrated the usefulness of the plant *Cuscuta reflexa* as analgesic, anti-inflammatory, antioxidant, hepatoprotective and anthelmintic agent.
This is a preliminary evaluation of the plant using suitable extracts of the plant. Many positive and useful indications were obtained from this study. It needs to be extended further to establish the phytoconstituents of this plant as a potential remedy for the treatment various diseases and disorders.

The future scope of this work includes:

1. Isolation and purification of the phytoconstituents like quercetin from this plant and detail biological screening of these phytoconstituents for the activity like anti diabetic, anti hypertensive, anti atherosclerotic etc.

2. The phytoconstituents can be subjected to various physicochemical standardization thereby a quantitative drug response relationship can be obtained.

3. The cytotoxic potential should be explored on full fledge basis using animal models and cell line studies on pure active constituents.