Psoriasis is a complex inflammatory skin disease in which epidermis becomes markedly hyperplastic and infiltrated with immune cells (mainly lymphocytes and monocytes). Pathophysiology of the disease associated with striking accumulations of superficial epidermal scale, hyperplasia and thickening of epidermis, exudation of inflammatory cells into dermis which contribute to the erythema, indurations, raised appearance and scaling. Immune cells are known to orchestrate the initiation and maintenance of pathophysiology of the disease. Immune cells cause release of excessive growth factors required for Keratinocyte proliferation, release of chemokine required for infiltration of more immune cells at the site, cytokine which regulate cellular immunity required for T cell activation and cell adhesion molecule expression. Although, it is now generally accepted that T lymphocytes play an important role in the maintenance of psoriasis but phenotype of psoriasis is dominated by abnormalities in Keratinocyte differentiation and proliferation.

Argemone mexicana (Mexican poppy) is considered as one of the traditional folk remedies for cancer, catarrh, chancre, cholecystitis, cold, colic, dysuria, eruptions, excrescences, eyes, fever, headache, herpes, inflammation, itch, ophthalmia, parturition, pink eye, rheumatism, tooth ache and many more. Argemone mexicana has also been reported effective against various skin disorders including psoriasis. However, mechanism of action for therapeutic values is yet to be explored with the pathophysiology of the disease studied so far.

In spite of the fact that Argemone mexicana has been known and widely used, the literature that identifies various medicinal uses of this plant provides no information to support the asserted effectiveness of the plant. The active components of Argemone mexicana responsible for medicinal activities are also unknown, though the literature reports the presence of alkaloids, flavonoids, amino acids, organic acids and sugars in the plant. Keeping in view the above clinical needs, present study is planned to characterize and establish toxicity, safety and pharmacokinetic profiling of Argemone mexicana with following objectives:
1. To characterize and study the profile of *Argemone mexicana*.
2. To obtain information concerning toxicity of *Argemone mexicana*, when administered in (i) Wistar rats (ii) Swiss mice; as a single dose by:
   (a) Intravenous and;
   (b) Oral Routes.
3. To characterize the profile of the extract following repeated exposure to determine the dose response relationship. The information derived from this study would also serve to indicate the possible toxicity including neurological, physiological, biochemical, hematological and exposure related morphological (pathological) effect likely to arise from repeated exposure of the test article and establish MTD (Maximum Tolerated Dose) and NOEL (No Observable Effect Level) dose, safety criteria for human exposure.
4. To evaluate local irritant effect on rabbit skin, following a single application of the plant extract.
5. To determine the degree of ocular irritation produced by the plant extract following a single instillation to rabbit eye.
6. To assess geno-toxicity potential by studying *in-vitro* chromosomal aberration induction potential in human lymphocytes.
7. To assess micronucleus induction potential in Swiss mice. The micronucleus test is a mammalian *in vivo* test, which detects damage to the chromosomes or the mitotic apparatus induced by test article/chemical.
8. To evaluate the photo-toxicity potential in Swiss mice.
9. To obtain the information regarding delayed dermal contact sensitizing (Hypersensitivity) potential after repeated exposure to Swiss mice and also to find out any adverse effect likely to occur due to dermal exposure of the extract of the plant.
10. To determine pharmacokinetic parameters and dose proportionality/linearity in wistar rats

Herbal formulation containing crude extracts of leaves of *Argimone mexicana* was evaluated in the proposed study plan. In the conduct of pre-clinical toxicological and pharmacokinetic investigations, standard methods were usually employed.
ACUTE TOXICITY

It is required to be carried out in two species usually, in mice and rats, as a single administration at predetermined dose(s) by the same route as intended for human.

In addition, at least one or more parenteral routes are used to ensure systemic absorption. Symptoms, signs, mortality and if possible causes of death are reported. Median lethal dose, i.e., LD₅₀ with 95% confidence limit is calculated. In case, LD₅₀ is not determined, then the reasons are stated.

REPEATED DOSE (LONG TERM) TOXICITY: In repeated dose study the drug is administered daily, 7 days a week, by intended clinical route. A vehicle treated control group and three other treatment groups in graded doses of test-article were selected. Since the proposed duration of medication in human is expected to be for three months, repeated dose toxicity studies have been conducted for 28 days and also for 180 days in rats.

The parameters to be mentioned and recorded in these studies include daily observation of clinical symptoms, mortality, weekly body weights and feed consumption, clinical chemistry, absolute and relative organ weights, urinalysis, gross pathological changes and histopathology. The studies establish No Observable Effect Level (NOEL) dose, Maximum Tolerated Dose (MTD) and target organ toxicity (if any) in rats. In addition to the above, Ames’ mutagenicity, immunotoxicity (MEST), phototoxicity, genotoxicity, local toxicity and toxicokinetic studies were also performed.

Acute toxicity of test article was assessed in Swiss mice and Wistar rats by a single oral administration. In both mice and rats, doses of 2000 and 5000 mg/kg were administered. No clinical signs, symptoms or mortality were observed during entire period of 14 days observation. No effect on body weight gain was observed in any treated group. On day-15, animals were sacrificed and gross pathology was recorded. Since there were no treatment related gross pathological lesions, histopathology was not done. Hence, LD₅₀ of test article, when administered orally as a single dose in both mice and rats was established as >5000 mg/kg.
Acute toxicity of test article was also assessed in Swiss mice and Wistar rats by a single intravenous administration. In mice, when doses of 500 and 1000 mg/kg were administered, mortality was observed as 0% and 50%. However in rats, a single initial dose of 1000 mg/kg had produced 50% mortality in treated group.

In mice, abdominal breathing and lethargy were observed up to 4 hrs. post dosing in low dose (500 mg/kg) group, however, high dose (1000 mg/kg) treated animals showed abdominal breathing, convulsions, prostration, sedation, lethargy followed by death. Surviving animals recovered completely within 24 hours and no abnormality was observed in any animal in the remaining period of observation, except sloughing of tails at the site of injection (few animals).

In rats, symptoms of abdominal breathing, prostration, lethargy and piloerection were observed in all animals immediately after dosing, however, symptoms of sedation and vasodilation were noticed in animals after about 2 hours. 50% mortality was observed within 24 hours of observation period. All surviving animals except one male showed sloughing of tail at the site of injection from day-7, while in female sloughing of tail at the site of injection was observed on day-6. On day-15, animals were sacrificed and gross pathology was recorded. Since there were no treatment related gross pathological lesions, histopathology was not done.

Median Lethal Dose (LD₅₀) of test article by intravenous route in both mice and rats was considered as =1000 mg/kg.

Repeated dose toxicity study was designed and conducted to assess toxicity in rats by daily oral administration of test article for 28 days with a view to obtain information on systemic or organ toxicity. A total of 80 wistar rats (40 males and 40 females) were divided into four groups of 10 males and 10 females each.

The rats were administered daily oral doses of test article for 28 days. The doses used were 0, 250, 500 and 1000 mg/kg/day. These animals were sacrificed after 28 days of dosing. No clinical symptoms that could be attributed to the medication of test article were noticed in any treated group. No mortality was noticed in any group during entire course of study.
Other study parameters recorded were weekly body weight gain, feed consumption, terminal urinalysis, ophthalmoscopy, hematology, clinical chemistry, absolute and relative organ weight analysis, gross and histopathology. Variations observed in above parameters and a few lesions found in gross and histopathology were comparable to those observed in control group animals. These were viewed as co- incidental and not treatment related. A significant increase in weights of liver and kidneys (all dose levels) and spleen (500 mg/kg dose only) was observed in male rats. In female rats, a significant increase in weight of liver (500 mg/kg dose only) and spleen (1000 mg/kg dose only) was observed. However, no increase in liver or kidney weights was observed in the 180 days repeated dose oral toxicity study in rats. The increase in liver and kidney weights in 28 days toxicity study may thus be due to adaptational changes in the body. In conclusion, the few adverse effects observed in rats treated orally with formulation of test article upto a dose of 1000 mg/kg/day could not be ascribed to the test article. Therefore, the MTD and NOEL by oral route is established as > 1000 mg/kg/day i.e. 20 times greater than proposed clinical dose*. *(Proposed Clinical Dose = 7 mg/kg/day. Hence, in rats it will be 7*7=49 mg/kg/day)

Similarly a 180 days repeated dose toxicity study was designed and conducted to assess toxicity in rats by daily oral administration of test article for 180 days with a view to obtain information on systemic or organ toxicity. A total of 280 Wistar rats (140 males and 140 females) were divided into four main groups of 30 males and 30 females and two reversal groups (control and high dose) of 10 males and 10 females each.

The rats were administered daily oral doses of test article for 180 days. The doses used were 0, 250, 500 and 1000 (Limit Dose) mg/kg/rat. The animals from main study groups were sacrificed after 180 days of dosing, however, reversal group animals were retained for further 30 days without dosing to evaluate the reversibility of toxicity, if any, found in main study group. No clinical symptom that could be attributed to the medication of test article was noticed in any treated group, however, two incidental mortalities were noticed in 500 mg/kg dose group during the entire course
of study. Other study parameters recorded were weekly body weights gain and feed consumption, interim/terminal urinalysis, ophthalmoscopy, hematology, clinical chemistry and terminal absolute & relative organ weight analysis, gross and histopathology.

A significant increase was observed in weights of adrenals (500 and 1000 mg/kg dose), heart (500 mg/kg) and spleen (500 mg/kg dose) in male rats. In female rats, a significant decrease in weights of adrenals (250 and 1000 mg/kg), and heart (1000 mg/kg) was observed. An increase in kidneys weights was observed at 500 mg/kg dose only. Thus, either these changes were not dose dependent and could not be attributed to test article administration.

Variations seen in above parameters and a few lesions found in gross and histopathology were comparable to those observed in control group animals, hence, these were considered as co-incidental and not treatment related.

In conclusion, no clear-cut dose-dependent adverse effects were observed in the rats treated orally with test article upto a dose of 1000 mg/kg/day. Therefore, the MTD and NOEL by oral route is established as > 1000 mg/kg/day i.e. 20 times greater than proposed clinical dose.

Genotoxicity of test article was evaluated by in vitro Chromosomal aberration and in vivo micronucleus tests in mammalian test system and Ames’ mutagenicity test. In Chromosomal Aberration Test, mitotic index in test article treated (with or without metabolic activation) and positive control groups was comparable to that of control group. However in Phase-I, cultures treated without S9 revealed 0.5%, 1%, 0% and 1% aberrations at 0, 6.25, 12.5 and 25 μg/ml concentrations, respectively. Percent aberrations observed in mitomycin C (Positive Control) treated cultures were 4, which were significantly higher than control. Culture treated with S9 revealed 0.5, 0.5, 1 and 0.5% aberration at 0, 6.25, 12.5 and 25 μg/ml concentrations respectively. Cylcophosphamide (Positive Control) treated group showed 5% aberrations, which was significantly higher than control and also proving sensitivity of the test procedure.
During Phase-II, cultures treated without S9 revealed 0, 0.5, 0.5 and 0.5% aberrations at 0, 6.25, 12.5, and 25 μg/ml concentrations respectively, while positive control group showed 5.5% aberrations and also proving the sensitivity of test system. Hence, test article was found to be **non-genotoxic** up to 25 μg/ml concentration in the test conditions employed. However, both the positive controls produced statistically significant genotoxic effect.

In **Micronucleus Test** the PCE:NCE ratio and incidence of micro-nucleated erythrocytes of various treatment groups were found to be comparable with that of vehicle control group, therefore, test article was found to be **non-genotoxic** up to 2000 mg/kg dose level. However, positive control (Mitomycin C) group exhibited statistically significant genotoxicity.

The **Bacterial Reverse Mutation Test** (Ames’ Test) was performed to evaluate test article for its ability to induce reverse mutation at the histidine locus in the genome of several strains of *Salmonella typhimurium* (TA97a; TA98; TA100; TA102 and TA1535) in the presence or absence of mammalian microsomal enzyme-S-9. It was found to be **non-mutagenic** in **Ames’ test** up to 5 mg/plate concentration of test article.

Test article was also found to be **non-irritant** in dermal and eye irritancy tests. It was also **non-phototoxic** at oral dose of 1000 mg/kg used daily for four days. In MEST it was found to be **non-sensitizing** up to 50% concentration of test article.

**PHARMACOKINETIC STUDIES IN RATS:**

**(A) ORAL ROUTE:**

The pharmacokinetic parameters were estimated using an indirect measurement (induction of IL-10) of test article at three dose levels in rats with single p.o. dose. The data did not show any gender-based differences at any of the dose levels.

Two peaks for $E_{max}$ values suggest that it could be due to absorption of different sets of compounds or metabolites, which are absorbed at different rate or at different sites in the gut or presence of active metabolites. The $E_{max}$ exhibited linear dose-proportionality to some extent.
Similar results were observed in the case of AUC<sub>0-t</sub>. In conclusion test article showed linear dose related pharmacokinetic parameters to some extent with E<sub>max</sub> and AUC<sub>0-t</sub>. No gender-based differences were observed.

**(B) INTRAVENOUS ROUTE:**

Two peaks were observed in male as well as female rats. The first peak was higher and was observed at 0.08 hr. The second peak was observed between 0.75-1.5 hr.

The second peak indicates redistribution of active ingredients in the body with passage of time or the presence of active metabolites. No dose proportionality of E<sub>max</sub> was observed in female and male rats. The AUC<sub>0-t</sub>, however exhibited linear dose proportionality. In conclusion two distinct peaks of E<sub>max</sub> were observed. The second peak may be due to redistribution of active ingredients in the body with passage of time or the presence active metabolites. The E<sub>max</sub> did not exhibit dose proportionality. However, linear dose proportionality was observed in the case of AUC<sub>0-t</sub>.

**CONCLUSION**

On the basis of the results obtained in above-mentioned pre-clinical toxicity and toxicokinetic studies, it is concluded that test article exhibits a very high safety margin. Hence, test article could be considered as a safe formulation for oral administration at proposed clinical doses.