

## STANDARDISATION OF HERBAL DRUG AS A POTENT LIVER TONIC

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### Summary

Liver disorders are serious health problems. In allopathic medicinal practices reliable liver protective drugs are not available but herbs play important role in management of liver disorders. Numerical medicinal plants are used for the same in ethnomedical practices and in traditional system of medicines in India. Many herbs were found in India as well as in tropical and sub tropical regions of the world.

Some Hepatoprotective function is achieved by herbal medicines based on phyllanthus species but these are declared as endangered plants by Indian Systems of Medicines. The present task was carried out to investigate the action of *Argemone mexicana* L plant material on CCl<sub>4</sub> (Carbon tetra Chloride) induced liver damaged in rats. Acute toxicity study, efficacy study, blood and tissue biochemical assays like ALT, AST, bilirubin, total protein, glucose, LM and EM etc have been studied for evaluation of Hepatocurative action.

**Keywords:** Hepatocurative, liver tonic, CTC, ALT, AST, LM, EM.

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### Introduction

*Argemone Mexicana* L. grows throughout in India as well as in tropical and sub tropical regions of the world. It is economically very important and is used as herbal medicine for many purposes. The root of this plant is diuretic and used in chronic skin diseases. The seeds are laxative, nauseant, emetic, expectorant and demulcent. The oil is purgative and also used for cutaneous affections. The injection of *Argemone* oil causes high tension, dropsy, diarrhea, vomiting and anaemia.

Liver disorder is one of the common thurst area declared by the Indian Council of Medical Research, New Delhi in the reviewed research on traditional medicine. In India the percentage of liver disorder is more as compared to developed countries, Phyllanthus species are common against such disorders (*P.amarus*, *P.asperalutus*, *P.dibilis*, *P. madaraspatensis* etc). Review and literature survey of some herbs were done which has ability to repair liver problems but many of them have some adverse problems.

### Materials and Methods:

*Argemone mexicana* L plant(Root Bark)s were collected from Western Ghats of Maharashtra; one of the hot spot of biodiversity and also from Pune District region, washed thoroughly and dried at room temp in shade for a week. They were powdered and stored in airtight containers. The herbarium sheet of this plant was prepared and authenticated from Botanical Survey of India, Pune. The powder of the plant material were sieved through standard BSS sieve of mesh 80/ 85 and preserved in airtight container.

### Dose:

The standard dose was decided for *Argemone mexicana* L root bark powder. The aqueous slurry is assigned in 0.01g/Kg body weight against CCl<sub>4</sub> damaged liver in rats, preciously for selection of amount of dose acute toxicity study has been also done in mice.

### Animals:

The animals used for study were procured from Raj Biotech Ltd. Pune and acclimatized for 15 to 20 days before study. They were housed in polymethane cages. Each cage housed with six animals and were maintained at 28<sup>±</sup>2<sup>0</sup>C. The animals were subjected to 12 hrs cycles of light and darkness. They were fed with commercially available Amrut feed pellets containing crude protein, crude fiber, calcium and phosphorus. Animals were supplied tap water from bottles during the experiment per day and the amount food and water intake is noted.

### Parameters Observed:

Blood of animals was collected by cardiac puncture under light ether anesthesia during sacrifice. Blood Biochemical assays were determined using a standard autoanalyser spectrophotometrically. The blood parameters observed were Alkaline Phosphates and Bilirubin, tissue parameters like Glucose, Total Protein and Cholesterol. All observations were statistically evaluated for significance using t-test and t-table.

### Animal Grouping:

Animals were grouped into five groups. Each group have 12 animals 6 males and 6 females for to compare sex dependant variables. Reversible liver damage was induced by 0.7 ml/Kg of CCl<sub>4</sub> in 0.5 ml. Liquid Paraffin per animal i.p. The dose of plant powder in the form of aqueous slurry was given orally via gavages as per dose decided (0.5g/Kg). Gr. I served as Normal Control, Gr. II served as CCl<sub>4</sub> Control, Gr. III served as CCl<sub>4</sub> Recovery, Gr. IV served as CCl<sub>4</sub> + Plant Slurry and Gr. V served as CCl<sub>4</sub> + silymarin (a known hepatoprotectant). The animals from all groups were sacrificed on 4<sup>th</sup> day and for of the study except the natural recovery group which was sacrificed on VII<sup>th</sup> day after natural recovery/ regeneration of liver was initiated.

### Dosage:

A reversible damage was induced in rat liver by administering low concentration of CCl<sub>4</sub>. The liver damage was induced by an intraperitoneally (ip) injection of CCl<sub>4</sub> (0.7 cm<sup>3</sup>/kg body wt) liquid Paraffin to each animal of group II to V

- An i.p injection of 0.5 cm<sup>3</sup> of liquid Paraffin was given to each animal from Gr. I as sham treatment.
- A standard dose in 0.5g/kg body wt of sieved herb/plant powder suspended in 2cc/distilled water were administered orally to each rat of Gr. IV
- A dose of 0.007-g/kg-body wt of silymarin (Silybon tablets manufactured by Ranbaxy lab. Ltd. India) suspended in 2CC of DW was administered orally to each rat of group V this dose is equivalent to the prescribed human dose of Silybon tablets.

## Results and Discussion

### Liver damage due to CCl<sub>4</sub>

Literature survey reveals that CTC causes hepatic injury and is a well-known liver toxin. CCl<sub>4</sub> has direct destructive effect on membranes of the hepatocyte and on consequent interface with cellular metabolism and transport. It damages the membranes of the hepatocyte causing leakage of the enzymes present in the cell. This results in elevation of the levels of plasma tramaminases.

It leads to fat decomposition in the liver due to blockage of secretion of hepatic triglycerides into plasma. The toxicity of CCl<sub>4</sub> depends upon the cleavage of C-Cl bond to generate a trichloro methyl- a free radical (CCl<sub>3</sub>O<sub>2</sub>). This cleavage occurs in the endoplasmic reticulum and is mediated by the cytochrome P-450 mixed function oxidase system. The product of the cleavage binds irreversibly to hepatic proteins and lipids. The metabolism of CCl<sub>4</sub> releases CCl<sub>3</sub> a free radical, which initiates per oxidation and cleavage of fatty acids in the membranes. The CCl<sub>4</sub> derived free radicals initiates the process of peroxidations by attacking Methylene Bridge of unsaturated fatty acid side chains of microsomal lipids. This results in early morphological alteration of endoplasmic reticulum and eventually to ultimate cell death through of series of changes listed below besides as yet underlined pathways like loss of activity of P450 xenobiotic metabolizing system, loss of glucose-b-phosphatase activity, loss of protein synthesis, loss of capacity of liver to form and excrete VLDL (Very Low Density Lipoproteins). Alterations in these parameters are used to monitor the course and extent of CCl<sub>4</sub> induced liver damage.

A single dose of CCl<sub>4</sub> leads to centrilobular necrosis and fatty liver. Within a few minutes, there is injury to the endoplasmic reticulum lending to functional defects of the Hepatocyte and multiple biochemical manifestations of hepatic injury. Irrespective of the route of administrations it leads to centrilobular necrosis and steatosis. Biochemical changes in the blood reflect injury. Serum enzyme levels increase with cytoplasmic enzyme reaching their peak within 12 hrs. Mitochondria enzymes reach their park within 36 hrs. Enzymes common to both mitochondria and cytoplasm reach their peak around 24 hrs.

CTC causes accumulations of fat in the liver especially by interfering with the transfer of triglycerides from the liver into the plasma. Many clinical conditions that cause an increase in cholesterol levels also cause increase in triglycerides enzymes sensitive to cytotoxic injury are serum glutamic pyruvic transaminase (SGPT) now called Alanine amino transferase (ACT) and serum glutamic oxaloacetic transferase (SGOT) now known as Aspartate amino transferase (AST). Aspartate and Alanine amino transferase are present in high concentration in liver.

Due to hepatocyte necrosis or abdominal membrane permeability, these enzymes are released from the cells and their levels in the blood increase. ALT is a sensitive indicator to acute liver damage and elevation of this enzyme in no hepatic disease is unusual. Alkaline phosphatase, although is not a liver specific enzyme, the liver is major source of this enzyme. Also the levels of this enzyme increase in cholestasis, elevated serum gamma-Glutamyl Transpeptidase levels appear to be indicative of diseases of the liver, biliary tract and pancreases. Bilirubin levels in blood also increase in liver diseases. (Cirrhosis and hepatitis).

### General Observations and Conclusions

**Observations of Biochemical parameters are as follows**

Parameter	Group I Normal Control	Group II CCl <sub>4</sub> control	Group III CCl <sub>4</sub> Recovery	Group IV CCl <sub>4</sub> + Plant Slurry	Group V CCl <sub>4</sub> + Silymarin
Cholesterol	82.00 ±0.00	93.2 ±1.20	92.00 ±1.02	86.10 ±1.30	90.1 ±1.30
AST	91.00 ±0.00	341.5 ±2.8	182.00 ±3.80	98.00 ±2.10	95.1 ±3.31
ALT	41.00 ±0.00	217.00 ±4.50	117.00 ±3.66	50.00 ±1.20	81.00 ±0.35
Alk.PO <sub>4</sub>	74.00 ±0.00	385.6 ±12.80	191.80 ±2.11	78.11 ±3.20	93.20 ±1.44
Bilirubin	0.54 ±0.00	0.86 ±0.10	0.80 ±0.12	0.59 ±0.20	0.64 ±0.20
Total Protein	82.42 ±0.00	266.92 ±14.20	174.31 ± 2.00	102.16 ±1.40	123.05 ±2.20
Glucose	92.29 ±0.00	230.57 ±8.00	145.12 ±2.00	103.40 ±1.30	143.26 ±1.50

The results obtained from blood biochemical parameters were considered logically. In clinical chemistry AST, ALT, Alkaline Phosphate, Bilirubin, total protein, glucose etc. values showed significant changes. All the values were compared with those of the control animals and the conclusions were obtained based on above observations.

Animals from all groups were observed for general and abnormal behavior, biochemical parameters and food and water consumptions. Observations of these animals were recorded and compared; the observations of Group IV were matched to Normal Control Group I as compare to Group V.

The observations of biochemical assay of Group I and Group IV (Plant Slurry Treated) showed good matching which indicates best recovery, Group II showed increased secretions indicating liver damage due to CCl<sub>4</sub>, Group III also showed more values of all biochemical parameters as natural recovery initiated and Group V showed some recovery due to Silymarin hepatoprotectant. The formulation was found equally effective in male as well as in female rats.

From above observations of biochemical parameters it was demonstrated that *Argemone mexicana L* indeed has a high potential in healing liver parenchyma and regeneration of liver cells hence it may act as a potent liver tonic.

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