

## Regenerating activity of *Citrus aurantifolia* on paracetamol induced hepatic damage

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Liver regulates many important metabolic functions. Hepatic injury is associated with distortion of these metabolic functions. Thus liver disease remains one of the serious health problems. In spite of tremendous studies in modern medicine, there are not much drugs available for the treatment of liver disorders. In the present study, paracetamol was used to induce the hepatic damage. Paracetamol, is a widely used analgesic and antipyretic drug. Oxidative stress is reported to play an important role in the pathogenesis of paracetamol – induced liver damage. The result confirm that *Citrus aurantifolia* possess a potential hepatoprotective activity.

Key words : Liver, paracetamol, *Citrus aurantifolia* and phytochemicals.

### INTRODUCTION

Liver is the largest gland in the human body and one of the most complexes of all human organs. It serves as the body main chemical factory and is one of its major store houses of food. The liver is a reddish – brown mass weighing about 3 pounds (1.4kg). It is located in the upper right part of the abdomen under the diaphragm and above the stomach and intestines.

The liver has a remarkable ability to produce new cells to replace its own diseased or damaged cells. For example surgeons can remove a section of healthy liver from an adult transplant it into a child who has a diseased liver. The adult's liver will rapidly regenerate and be restored to full size. The child's new liver will grow as the child grows (Lewis and Zimmerman, 1989).

The blood carries these nutrients as well as vitamin, minerals and fatty acids and glycerol to the liver. The liver removes the excess glucose from the blood and stores it in the form of a starch like compound called glycogen. Glucose serves as the chief fuel for the body's cells. When the body needs energy the liver convert's glycogen to glucose and releases it into the blood, the liver also converts fatty acids and amino acids into glucose when its storage of glycogen is low (Jones *et al.*, 1996).

The liver also plays an essential role in the storage of certain vitamins. The liver stores vitamin A as well as vitamin D, E and K and those of the B complex group. It also stores iron and other minerals. Liver cells filter harmful substances from the blood. Such substances include insecticides, drugs, food additives and industrial chemicals. Enzyme in the liver cells converts some of

these substances into products that dissolve in water (Werth *et al.*, 1993).

The juice of the lime is regarded as an antiseptic, tonic, an antiscorbutic, an astringent, and as a diuretic in liver ailments, a digestive stimulant, a remedy for intestinal hemorrhage and hemorrhoids, heart palpitations, headache, convulsive cough, rheumatism, arthritis, falling hair, bad breath, and as a disinfectant for all kinds of ulcers when applied in a poultice. The leaves are poultice on skin diseases and on the abdomen of a new mother after childbirth. The leaves or an infusion of the crushed leaves may be applied to relieve headache. The leaf decoction is used as eye drops and to bathe a feverish patient; also as a mouth wash and gargle in cases of sore throat and thrush. The root bark serves as a febrifuge, as does the seed kernel, ground and mixed with lime juice. In the West Indies, the juice has been used in the process of dyeing leather. The dehydrated peel is fed to cattle. In India, the powdered dried peel and the sludge remaining after clarifying lime juice are employed for cleaning metal. The hand-pressed peel oil is mainly utilized in the perfume industry. Lime juice dispels the irritation and swelling of mosquito bites.

In the present study paracetamol was chosen to induce liver damage for evaluation of hepato protective activity. To assess the MDA, GSH content, SGOT, SGPT activity, Albumin level, protein content, vitamin E and Vitamin C to evaluate the liver damage of the above biochemical parameters are used to assess the hepatoprotective activity of plant.

## MATERIALS AND METHODS

### **Plant materials and drug preparation:**

Dried xylem of *Citrus aurantifolia* was collected from our house garden at Thanjavur. The *Citrus aurantifolia* shade dried and finally powdered which was sieved through nice cloths and used as drug. The fine powder was dissolved in distilled water just before oral administration.

### **Experimental design:**

The male albino rats were kept in polypropylene cages under standard environmental conditions (at an ambient temp of  $25 \pm 1^\circ\text{C}$  and 45-55% Relative Humidity with a 12:12 hour light / dark cycle). Experiments were conducted between 09.00 and 14.00hrs (Local Animal Ethical Committee of Dept. of Pharmacology, Periyar College of Pharmacy for Women, Trichy. Animal Ethical Number: CPCSEA/265). Drugs were administered orally through the sterile syringe. Body weight of animals were recorded and they were divided into three groups of 6 animals each as follows

Group I: Normal animals received with standard fed and water to allow ad libitum.

Group II: One administration of paracetamol (3kg b/wt) for single dose

Group III: Paracetamol administration was done for single dose. Treatment was started (after 24hrs of induction) by oral administration of *Citrus aurantifolia* at a dose of 500mg/dl b/wt for 10 days.

After the completion of experimental regimen, that rats were fasted overnight and blood samples were collected by the puncturing the retro orbital plexus under light either anaesthesia. Serum was used for the analysis of various biochemical parameters such as Protein (Lowry *et al.*, 1951), Serum Glutamate Oxaloacetate Transaminase (SGOT; Reitman and Frankel, 1957), Serum Glutamate Pyruvate Transaminase (SGPT; Reitman and Frankel, 1957), Albumin (Rodkey, 1965), Malondialdehyde (MDA; Beuge and Aust, 1978), Glutathione (GSH; Moron *et al.*, 1979), Vitamin C (Omaye *et al.*, 1979), Vitamin E (Baker *et al.*, 1980).

### **Statistical analysis:**

The result were present as mean  $\pm$  SD. Data were statistically analyzed using student 't' test. P. values set as lower than 0.001, 0.01, 0.05 were considered as statistically significant.

## RESULTS AND DISCUSSION

The present study was carried out to evaluate the *Citrus*

*aurantifolia* on paracetamol induced liver injury. The observations made on different groups of experimental and control animals were compared as follows. Table 1 represents the level of MDA, GSH, SGOT, SGPT, Protein, Vitamin C, Vitamin E, albumin in serum of normal and experimental rats.

Group II paracetamol intoxicated rats showed a significant increase in the level of MDA, SGOT, SGPT when compared to group I rats. Group III paracetamol intoxicated rats treated with *Citrus aurantifolia* showed significant decrease in the level of MDA, SGOT, SGPT when compared to group II.

Group II paracetamol intoxicated rats showed a significant decrease in the level of GSH, protein, vitamin C, Albumin when compared to group I rats. Group III paracetamol intoxicated rats treated with *Citrus aurantifolia* showed significantly increase in the level of GSH, protein, vitamin C, Albumin as compared to group II.

Paracetamol (N-acetyl p – aminophenol, acetaminophen) a widely used analgesic and antipyretic drug is known to cause hepatotoxicity in experimental animals and humans at high doses, it is mainly metabolized in the liver excretable glucuronide and sulphate conjugates however, hepatotoxicity of paracetamol has been attributed to formation of toxic metabolites when a part of paracetamol is activated by hepatic cy<sup>+</sup> P450 to a highly reactive metabolite N-acetyl-p-benzo quinoneimine which is normally conjugated with GSH and excreted in the urine as conjugates. Overdose of paracetamol leads to mitochondrial dysfunction followed by acute hepatic necrosis (Kumar *et al.*, 2005).

SGPT present in large concentration in the liver and in fewer amounts in kidney and it is more specific for liver disease than SGOT. This test is highly sensitive but it is also very non – specific because it defects only that there is a liver damage, giving a few information about liver disease that cause. SGPT levels are generally higher than SGOT level when a liver damage is present (Malon, 2004).

The study of lipid peroxidation is attracting much attention in recent years due to its role in disease process. Lipid peroxidation (LP) has been implicated in the pathogenesis of number of disease and clinical conditions. They include atherosclerosis, cancer, respiratory disease syndrome, alzheimer's disease, Parkinson's disease chemical and radiation induced injury etc. Experimental and clinical evidence suggests that aldehyde products of LP can also act as bioactive molecules. In physiological and pathological conditions it is now generally accepted that LP and its products play an important role in liver,

**Table 1 : Effect of *Citrus aurantifolia* on MDA, GSH, SGOT, SGPT, Protein, Vitamin C, Vitamin E, albumin in experimental rats**

Parameters	Group I	Group II	Group III
MDA (mg / dl)	2.98 ± 1.54	11.57±2.49*	3.85±2.63**
GSH (mg/dl )	67.93±7.68	40.63 ± 5.86*	59.68±8.89**
SGOT (IU / dl)	33.66 ± 2.13	62.52±5.18*	33.66±1.83**
SGPT(IU/dl )	24.49±1.40	42.71 ± 1.34*	24.53±1.37**
Protein (gm /dl)	6.24 ± 0.95	4.79±0.65*	6.16±0.45**
Vitamin C(mg/dl)	40.79 ± 4.05	26.58 ±2.43*	40.23±3.80**
Vitamin E (mg/dl)	6.12 ± 1.02	5.19 ± 0.41*	6.23 ± 1.16 **
Albumin (gm/dl)	5.11 ± 1.44	2.10 ± 0.98*	4.55± 1.66**

Values were expressed as mean ± SD for six rats in each group

\* Significantly different from group I

\*\* Significantly different from group II

kidney and brain toxicity (Dryden *et al.*, 2005).

In the present study, elevated level of LPO in paracetamol treated rats is a clear of excessive formation of free radical and activation of lipid peroxidation system resulting in hepatic damage. The significant decline in the level of these constituents in paracetamol and *Citrus aurantifolia* treated animals indicated antilipid peroxidative effect of *Citrus aurantifolia*.

GSH is a ubiquitous thiol containing tripeptide, which plays a central role in cell biology. It is implicated in the cellular defence against xenobiotics and naturally occurring deleterious compounds such as free radicals. Glutathione status is a highly sensitive indicator of cell functionality and viability perturbation of GSH status of biological system has been reported to lead to serious consequences (Udaybandyopadhyay, 1999). Decline in GSH content is paracetamol-intoxicated rats and its subsequent return towards near normally in paracetamol and *Citrus aurantifolia* treated rats reveal antioxidant effect of *Citrus aurantifolia*.

The liver synthesizes almost all the plasma proteins except immunoglobulins. Hence, the measurement of serum proteins forms a reliable index of liver function. Serum total protein and albumin are quantitatively the most important protein synthesized by the liver and reflects the extent of functioning liver cell mass. Chronic diseases of the liver generally lower serum total protein and albumin concentrations, hypo proteinemia (Kaplan, 1993).

In the present study, significantly decreased level of protein and albumin were observed in paracetamol-intoxicated rats. Restored the protein and albumin level treated with *Citrus aurantifolia* the restorative activity of *Citrus aurantifolia* on protein and albumin indicated the well organized hepatic synthesis.

An antioxidant has been defined as “and substance that when present of low concentration. Compared with those of an oxidizable substrate significantly delays or

prevent oxidation of substrate. When ROS/RNS are generated in view, their actions are opposed by intricate and coordinated antioxidant lines of defense system. This includes enzymatic and non - enzymatic antioxidant that keeps in check ROS/ RNS level and repair oxidative cellular damage (Halliwell and Gutteridge, 1999) circulatory non - enzymatic antioxidant such as vitamin E and vitamin C are free radical scavengers. Their synergistic action in scavenging oxygen derived free radicals is well documented (Wojacki *et al.*, 1995) vitamin E reacts with lipid peroxy radicals acting as a chain terminator of lipid per oxidation while vitamin C helps to maintain the level of vitamin E at optimum concentration. Serum level of vitamin E and vitamin C in the present study were significantly reduced in *Citrus aurantifolia* as compared with control.

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