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Research Article

## Hepato protective activity of *Cucumis sativus* L.

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### ABSTRACT

Liver disorder is one of the familiar health troubles and it is exposed to several category of xenobiotics and therapeutic agents. The present research was designed to investigate the possible Hepatoprotective effect of Ethanolic extract of *Cucumis sativus* L. (EECS) against the paracetamol-induced hepatotoxicity. The EECS at 150 mg/kg, p.o. showed a significant hepato protective activity against, paracetamol-induced hepatotoxicity as judged by measuring levels of serum marker enzymes like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP). The serum levels of total proteins and bilirubin were also estimated in treatment of rats with dose level of 150mg/kg of plant extract. Significantly ( $P < 0.001$ ) changed serum marker enzymes levels closer to normal against paracetamol treated rats. The activity of the EECS at dose of 150 mg/kg was nearly comparable to the standard drugsilymarin (25 mg/kg, p.o.). A histopathological study of liver exhibited almost normal architecture, when compared to paracetamol treated group. The present study showing that EECS has significant hepato protective effect against paracetamol-induced hepato toxicity in rats

**Keywords:** *Cucumis sativus* L.; SGOT; SGPT; Paracetamol; Silymarin

### INTRODUCTION

It is well documented that the liver is an essential organ, engaged in the maintenance of metabolic job and detoxification from the endogenous and exogenous face, like drugs, xenobiotics, viral infections and alcoholism (MossaJS et al. 1991). Sufficient supply of blood and presence of many cytochromes and various enzymes available in liver convert these materials into different types of inactive, active or toxic metabolites (Mascolo N et al. 1998, Goldin et al. 1996). And also serum levels of many biochemical markers like SGOT, SGPT, bilirubin and Total Protein are elevated (Hinson et al. 2002).

Around 25, 000 deaths occur every year owing to liver disorders (Jollow JR et al. 1973). Liver damage is caused by different origins, such as alcohol, chemicals, viruses, and auto-immune diseases (Potter WZ et al. 1973). Paracetamol, used widely as a counter analgesic and antipyretic drug, and frequently used in alleviate of fever, headaches. It was accounted that large doses of paracetamol may produce serious liver necrosis in humans and in experimental animals.

Paracetamol is commonly metabolized in the liver through glucuronidation, oxidation and sulfonation (Dahlin DC et al. 1984). The sulfonation and glucuronidation are very important metabolic reactions than compared to oxidation, but oxidation is the major cause as far as toxicity is concerned (Sharma K et al. 2010). Oxidation of paracetamol is principally catalyzed by cytochrome P-450 (Sugiyama K et al. 1999) and create a more reactive arylating compound named as N-acetyl-p-benzoquinoneimine (NAPQI).<sup>10</sup> In the human liver microsome P-4501A2, is the principal catalysts of paracetamol (Hinson JA et al. 1980).

Semi-quinone radicals, acquired by one electron reduction of NAPQI is quickly conjugated with GSH and it is released as the cysteinyl conjugate or mercapturic acid (Mitchell JR et al. 1988). According to the rate of formation of NAPQI is not more than the maximal rate of synthesis of GSH there will be nontoxic effect to the cell or organ.

But, excessive intake of paracetamol produce hepatotoxicity induced by the reaction metabolite N-acetyl-P-benzoquinoneimine (NAPQI). In absence of a dependable liver protective drug in the current system of medicine, a number of medicinal remedies are available in herbal treatment. The Indian herbal medicine system, are suggested for treatment of liver disorders (Chatterjee TK et al. 2000). Natural therapies from medicinal plants are measured by its effective and safe alternative treatments for hepatotoxicity. On considering the above statement, the present study was undertaken to explore the hepatoprotective activity of *C. sativus*

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leaves against paracetamol induced hepatotoxicity in Albino rats.

## MATERIALS AND METHODS

### Drugs and Chemicals

All reagents used in the procured were analytical grade.

Paracetamol tablet (Sun Pharmaceuticals Ltd) purchased from a drugstore. -Total Bilirubin, Direct Bilirubin, Total Proteins, SGOT, SGPT, Alkaline Phosphate was assayed by using kits from Ranbaxy diagnostic, New Delhi.

### Plant collection

Fresh leaves of *C. sativus* was collected from field of Komarapalayam and authenticated by Dr.P. Satyanarayana, Scientist D & Head office in charge, Southern Regional centre, TNAU campus, Coimbatore. Voucher specimen (No: JKKNC/0102/ 13) has been deposited in the Department of Pharmacognosy, JKK Nataraja College of Pharmacy, Komarapalayam, Tamilnadu, India.

### Preparation of Plant Extracts

The dried leaves are extracted with alcohol and then alcoholic extract of each plant was subjected to solvent extraction.

### Ethanol extract of *C. sativus* Linn (EECS)

Fine powdered Leaves of *C. sativus* was extracted successively with petroleum ether and Ethanol (60-80° C) using soxhlet apparatus. The extract was filtered and evaporated to separate solvent and residue. The semi-solid residue thus obtained was stored in desiccators until further use.

### Animals

Albino rats either sex weighing between 175 ± 25gm were used in this evaluation. These rats aged between 2 - 2.5 months were procured from animal house located in JKK Nataraja College of Pharmacy, Komarapalayam. They were housed in well ventilated stainless-steel cages at room temperature (24±2°C) in hygienic condition under natural light and dark schedule and were fed on a standard laboratory diet. Food and water were given ad libitum.

### Experimental protocol

#### Acute oral toxicity study

Acute oral toxicity study was followed by using OECD GUIDELINES - 423 (Organization of Economic Cooperation and Development) - Fixed dose procedure (FDP) Acute toxicity study was performed for EECS according to the acute toxic classic method as per OECD (423) guidelines<sup>5</sup>, albino rats were used for acute toxicity study. The animals were kept in fasting condition for overnight providing only water, then the extract was administered orally at the doses of 5, 50,

300 and 2000 mg/kg and observed for 16 days. If death was observed in 2 out of 3 animals, then the dose administered was concluded as toxic dose. Animals aren't shown signs of toxicity including mortality, nature, severity, and duration of effects up to the dose level of 2000 mg/kg for the extract.

**Hepatoprotective Activity:** (Neharkar S et al. 2010, Praveen TK et al. 2009)

Group 1- Normal control rats, which received 0.5% Carboxy methyl cellulose (CMC) solution (1ml/kg) one time daily for 7 Days.

Group 2- Hepatotoxient, administered with paracetamol (3gm/kg) a single dose on day 7.

Group 3- Standard drug control, receives silymarin (100mg/kg) once daily for 7days (Std).

Group 4- Receives EECS (150mg/kg) once daily for 7 days.

Group-3 and 4 receives paracetamol (3gm/kg) as a single dose on 7<sup>th</sup> day, after thirty minutes administration of drug extract and silymarin respectively.

**Assessment of Hepatoprotective Activity:** (Stater TF et al. 1965, Ashok SK et al., 1991)

In the present study the hepatoprotective activity was evaluated biochemically and histopathologically. After 24 hours of drug treatment, the animals were dissected under ether anaesthesia. From each rat the blood sample was withdrawn from the carotid artery in the neck and collected in previously labelled centrifuging tubes and allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 7000 RPM for 10 minutes. The separated serum was used for the estimation of some biochemical parameters like SGPT, SGOT,, bilirubin and protein.

For the histopathological study, liver from each animal was removed after dissection and preserved in 10% formalin. Then representative blocks of liver tissues from each lobe were taken and possessed for paraffin embedding using the standard microtechnique. Sections (5 µm) of livers stained with eosin and hematoxylin, observed microscopically for histopathological studies.

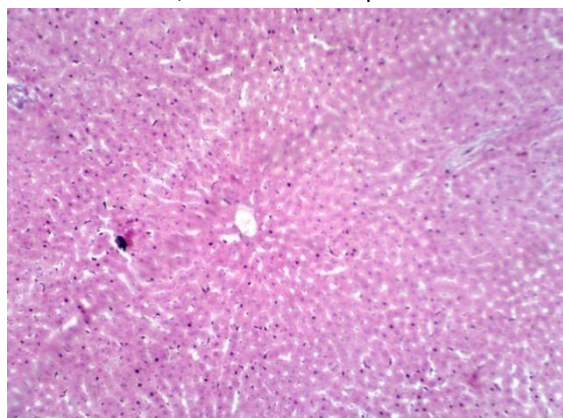
### Results and discussion

Paracetamol induced hepatotoxicity is selected as a screening method for testing hepatoprotective nature of EECS. The previous reports of paracetamol toxicity in human reporting about hepatic necrosis (Galighor AE et al. 1976, Davids on DGD et al. 1966, Pimston BL et al. 1968). It is functioning as an experimental hepatotoxic agent. Paracetamol toxicity is due to the formation of the toxic metabolites when it is metabolized by enzyme cytochrome P450 (Boyers TD et al. 1971). and forming a minor significant alkylating metabolite named as NAPQI. NAPQI is then permanently conjugated to the sulfhydryl groups of glutathione (lb-

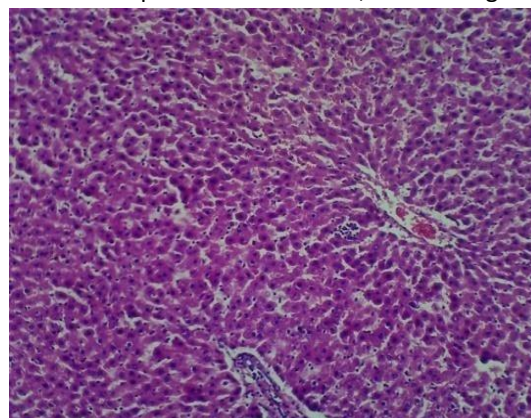
**Table 1: Hepetoprotective activity of EECS**

S.NO	Particulars	SGPT (U/ml)	SGOT (U/ml)	ALP (U/ml)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Total Protein (mg/dl)
1.	Control	63.73±5.33	135.03± 12.21	170.33±22.22	0.653±.13	0.31±0.17	6.98±0.19
2.	Paracetamol (3gm/kg)	282.58±10.13*	419.65±25.93*	436.51±27.07*	2.14±.90*	1.89±0.12*	2.56±0.24*
3.	Sylimarin (100 mg/kg)	68.31±7.44†	171.06±17.75†	175.88±22.84†	0.94±.17†	0.34±0.16†	5.87±0.25†
5.	EECS (150 mg/kg)	174.08±20.92†	222.7±14.06†	206.30±17.47†	1.45±.31 <sub>ns</sub>	0.85±0.10†	4.73±0.29†

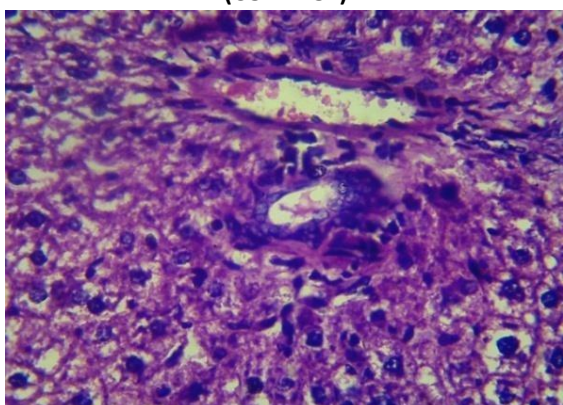
Values are expressed as mean ± S.D. of six animals in each group. Statistical analysis ANOVA followed by Dunnett-test. N= 6; \*P <0.01 as compared with control, †P <0.01 as compared with standard, ns = non significant



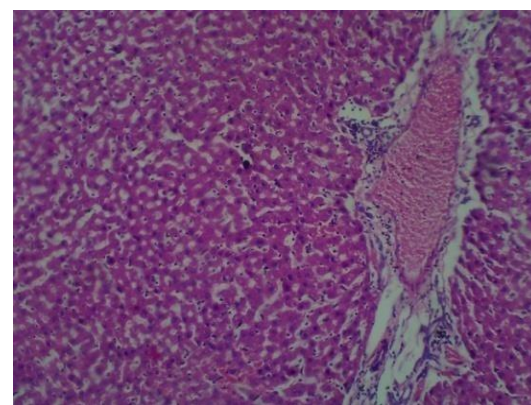
**Figure 1: Histopathology of Liver sections GROUP I (CONTROL)**



**Figure 2: GROUP II(PARACETAMOL INDUCED)**



**Figure 3: GROUP III (SYLIMARIN)**



**Figure 4: GROUP IV (EECS)**

rahim M *et al.* 2011). NAPQI depletes glutathione and start covalent binding to cellular proteins. These events lead to the disturbance of calcium homeostasis, oxidative stress, mitochondrial dysfunction, and also lead to eventually culminate in cell damage and death. An understandable symbol of hepatic injury is the escape of cellular enzymes into the plasma (Schmidt E *et al.* 1975) was due to the disturbance take place in the transport of hepatocytes (Zimmerman HJ *et al.* 1970). When liver cell plasma damaged, a number of enzyme located in cytosol is released into the blood, hence increased enzyme levels found in the serum.

Supporting of the above mechanisms, in the present study the EECS reduce the level of proteins and increase the level of serum marker enzymes widely (Table 1).

**Histopathology**

Histopathological side view also exposes a major damage in the same groups. The observable fact is proved by liver biopsy (Fig. 1 to 4). The liver cell necrosis, especially in the centrilobular, loss of cell boundaries and hepatic manner, and ballooning collapse, were well-known in the sections of liver injected with paracetamol at a dose of 3 mg/kg (Fig. 2). In the standard group, i.e. treatment with sylimarin (100mg/kg.), the hepatic manner (Fig. 3) was similar to that observed in the vehicle control (Fig. 1). While administration with EECS (150 mg/kg) repaired a large portion of liver damaged by paracetamol (Fig.4), there were only slight necrosis, a reduced degree of ballooning, fatty accumulation, infiltration of the lymphocytes around the cen-

tral vein. Report shows flavonoids and triterpenoids may be responsible for hepatoprotective effect in the plant.

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