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# An Improvement in Hepatoprotective Activity by Herbal Drug Combination

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**Abstract:** The medicinal plant of *Trichosanthes cucumerina* L. (F.Cucurbitaceae) are used to treat liver disorders. It is one of the ingredients in various Ayurvedic formulations used especially for the treatment of liver disorders and also in other diseases. The purpose of the this study is to evaluate the hepatoprotective activity of ethanol extract of *cucumerina* (EETC) alone and Mixture of Ethanol extract of *cucumerina* and *Coriandrum sativum* (MEETC &EECS) was carried out in Male Wistar rats. Liver damage in rats were induced by intraperitonial administration of Paracetamol (3gm/kg,b.w,p.o) for 3 days. An ethanol extract of *cucumerina* (EETC (150mg/kg,) and in other group Mixture of ethanol extracts of *cucumerina* (EETC) with *Coriandrum sativum* (MEETC&EECS 1:1 ratio and 150mg/kg) was given for 7 days. Silymarin ((100mg/kg, p.o) was used as a standard drug. The following parameters are analyzed by collecting blood from direct cardiac puncture under light (viz Total Bilirubin, Indirect Bilirubin, Direct Bilirubin, Total Proteins, Albumin, Globulin, Serum Glutamic-Oxaloacetic Transaminase(SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) and Serum Alkaline Phosphatase (ALP). MEETC&EECS shows significant activity when compared to EETC alone. It was confirmed by histopathological study also. The effect of extract 150mg/kg was almost comparable to the standard drug.

**Key words:** *Trichosanthes cucumerina* L. · *Coriandrum sativum* L. · Paracetamol · Hepatoprotective · Silymarin · EETC · MEETC&EECS

## INTRODUCTION

The liver is one of the major organs in human body which plays an important role in intensifying metabolism and excretion [1, 2] and it has an amazing role in the preservation, performance and regulating homeostasis of the body. It is occupied almost all the biochemical pathways of development, fight against all diseases, nutrient supply, reproduction [3] and energy provision. The liver is commonly not only to carry out physiological functions, but also to shield against the toxic effect of harmful drugs [4]. Due to wonderful efficient development in the field of hepatology, liver problem is one of are on the rising problem in recent years. Drug-induced liver injury is a major health problem take place in health care professionals, pharmaceutical industry and drug regulatory agencies. The United States Acute Liver Failure Study Group, survey referred that 50% of acute liver failure take place by including hepatotoxicity caused by overdose of Paracetamol (39%) and idiosyncratic liver injury triggered by other drugs (13%) [5]. Drugs play an vital role in cause of liver injury. Some surveys indicate that approximately 75% of the idiosyncratic drug reaction results in liver transplantation or even death [6]. It also refer that the rate of hepatotoxicity reported that are much higher in developing countries like India (8% - 30%) compared to that in advanced countries (2% - 3%) with a similar dose schedule [7]. In spite of incredible advanced in modern medicine, there are hardly any reliable drugs that protect the liver from damage and help in regeneration of hepatic cell. Many active plant extracts are recurrently employing to treat a wide range of diseases including liver disease. Herbal medicines are believed to be much safer and proved elixir in the treatment on various ailments [8]. Therefore, searching for effective and safe drugs for liver disorders are continuing to be an area of interest.

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*Cucumerina* is a well known plant (F. Cucurbitaceae) generally known as snake gourd, snake tomato, viper gourd or long tomato. The fruit is consumed as a vegetable because it contains a good nutritional value [9]. The plant is richly composing of flavonoids, phenolic acids, carotenoids and triterpinoids. It has an important role in alternative systems of medicine like Ayurveda and Siddha due to its various pharmacological activities like hepatoprotective, antidiabetic, cytotoxic, anti inflammatory and larvicidal effects [10].

*Cucumerina* is also used traditionally in the treatment of headache, fever, abdominal tumors, alopecia, bilious, jaundice acute colic, boils, diarrohea, haematuria and skin allergy, vermifuge, stomachic, refrigerant, purgative, malaria, laxative, hydragogue, hemaglutinant, emetic, cathartic, bronchitis and anthelmintic[11].

## MATERIALS AND METHODS

### Drugs and Chemicals

**All Reagents Procured Were Analytical Grade:** Paracetamol tablet (Sun Pharmaceuticals Ltd) purchased from a drugstore. Total Bilirubin, Total Proteins, SGOT, SGPT, Alkaline Phosphate were assayed by using kits from Ranbaxy diagnostic, New Delhi.

**Plant Collection:** Fresh leaves of *cucumerina* L. and *sativum* fruits were collected from Komarapalayam and authenticated by Dr.P. Satyanarayana, Scientist D & Head office in charge, Southern Regional Centre, TNAU campus, Coimbatore. The leaves were cut into small pieces and air dried indoor subdued light and with good ventilation and then crushed into a fine powder using a laboratory Homogenizer, which passed through 22 No sieve.

The fruits of *sativum* were dried and then crushed into fine powder by using laboratory homogenizer then stored for further use.

### Preparation of Plant Extracts

**Ethanol Extract of Cucumerina Linn. (EETC):** Fine powdered Leaves of *cucumerina* were 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 semisolid residue thus obtained was stored in desiccator until further use.

**Ethanol Extract of Sativum: (EECS):** Fine powdered fruits of *sativum* were extracted successively with petroleum ether and ethanol (60-80°C) using Soxhlet apparatus in muslin cloth packed column. The extract was filtered and evaporated to separate solvent and residue. The semisolid residue thus obtained was stored in desiccator until further use.

**Mixture of Ethanol Extract of Cucumerina Linn with Sativum: (MEETC&EECS):** Ethanol extract of *Cucumerina* L. and Ethanol extract of *Sativum Linn* were taken in equal proportion and dissolved in 0.5% Carboxyl Methyl Cellulose and used as MEETC&EECS.

**Animals:** Male Wistar rats weighing 250 ± 20g were used for hepatoprotective studies and albino rats used for acute toxicity studies. The animals were kept in polypropylene cages and maintained at 25 ± 5°C less than 12 h light/dark cycle. The animals were permitted for free access standard pellet diet and water *ad libitum.*

### Experimental Protocol

**Acute Oral Toxicity Studies [12, 13]:** The acute oral toxicity study was followed by using OECD GUIDELINES - 423 (Organization of Economic Co-operation and Development) - Fixed dose procedure (FDP).

Acute toxicity study was performed for ethanol extract of *Cucumerina* L. (EETC) and mixtures of ethanol extract of *Cucumerina* L. with ethanol extract of *Sativum* (MEETC&EECS*)* extract according to the acute toxic classic method as per OECD (423) guidelines5, 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 dose of 50, 100, 200 and 500 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. No death was occurring up to the dose level of 500mg/kg.

### Hepatoprotective Activity [14-21]:

*Group 1*: Normal control, received 0.5% Carboxy methyl cellulose (CMC) solution (1ml/kg) once daily for 7 Days.

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

*Group 3*: Receives Trichosanthes cucumerina extract (150mg/kg) once daily for 7 days. (EETC).

*Group 4*: Receives combination of ethanol extract of Trichosanthes cucumerina extract and coriandrum sativum extract in 1:1 ratio(150mg/kg) for 7 days. MEETC&EECS.

Table 1: Effect of Extracts in Paracetamol induced hepatotoxicity rates

| Parameters | Control | Paracetamol | EETC | EETC&EECS | Standard |
|---|---|---|---|---|---|
| BILIRUBIN(d/l) | 0.31±0.17 | 1.89±0.12*** | 0.79±0.13*** | 0.35±0.27*** | 0.34±0.16*** |
| SGOT (U/l) | 135.03±12.21 | 419.65±25.93*** | 218.83±14.12*** | 195±14.75*** | 171.06±17.75*** |
| SGPT (U/l) | 63.73±10.94 | 282.58±10.31*** | 138.11±10.96*** | 79.5±17.78*** | 68.31±7.44*** |
| ALP (U/l) | 170.33±22.22 | 436.51±27.07*** | 226.10±17.39*** | 198.5±30.02*** | 175.88±22.84*** |

*p < 0.05; ** p < 0.01; *** p < 0.001.

Values are expressed as mean (standard deviation; SD). Statistical significance was calculated with ANOVA followed by tukey compare all pair of coloum treated group with paracetamol group

*Group 5*: Standard drug control, receives silymarin (100mg/kg) once daily for 7 days (Std).

Group 3 to Group 4 receives paracetamol (3gm/kg) as a single dose on day 3, Thirty minutes after administration of drug extract and silymarin respectively.

**Assessment of Hepatoprotective Activity:** All the extracts and the paracetamol were administered orally by suspending in 0.5% CMC solution. Animals were sacrificed under light ether anesthesia 24-h after the last dose. Blood was collected through cardiac puncture in plain tubes and liver was removed, rinsed in cold saline, blotted with filter paper and weighted. 10% (w/v) liver homogenate was prepared in 0.25M sucrose solution and centrifuged at 7000 rpm for 10 min at 4°C.

When serum clearly separated out, the serum was analyzed for Serum Glutamic-Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) and Serum Alkaline Phosphatase (SALP) levels using a reagent kit by the method proposed by Reitman and Frankel. The results thus obtained were subjected to statistical analysis using student t-test and analysis of variance [17]. The report of serum was illustrated in the Table 1. For the histopathological study, liver from each animal was removed after dissection and preserved in 10% formalin solution. Then representative blocks of liver tissues from each lobe were taken and processed for paraffin embedding using the standard microtechnique [18]. Sections (5 µm) of livers stained with hemotoxylin and eosin, were observed through a microscope.

**Histopathological Studies [22-26]:** The light microscopy examination of the transverse section of paracetamol treated and extract treated mice livers were shown in Figures 1 to 5. Figure 2 shows the liver of paracetamol intoxicated mice shows a wide necrosis across the cells. The liver sections of the paracetamol intoxicated mice showed necrosis, ballooning and degeneration in the hepatic plates and loss of the cellular boundaries and karyolysis. Accumulation of neutrophils also found.

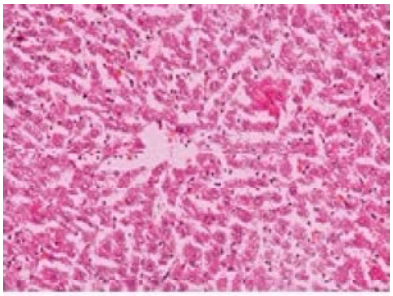

Fig. 1: Section showed that the structure of liver of normal control. The portal traids, hepatocytes, hepatic sinusoids and central veins appear normal. There is no kuffer cell hyperplasia-normal histological appearance

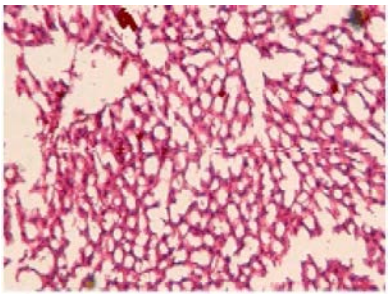

Fig. 2: Section showed that the structure of liver of paracetamol induced rat. The hepatocytes around the central veins (Zone 3) show fatty change and tranucleareosinophilic inclusions. The zone 1 and zone 2 hepatocytes appear normal

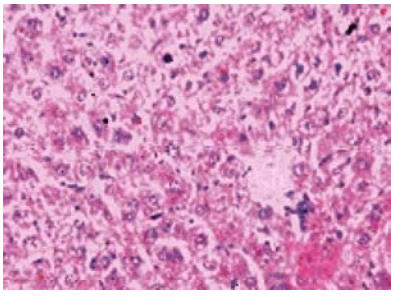

Fig. 3: Section showed that the structure liver of EETS. The portal tracts and the periportal hepatocytes (Zone 1) appear normal. The zone 3 hepatocytes show fatty change and hepatocellular necrosis

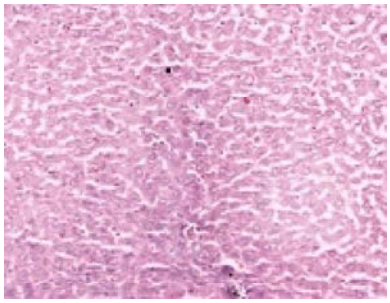

Fig. 4: Section showed that the structure liver of EETS&EECS. The hepatocytes in all the 3 zones appear normal. There is no evidence of hepato cellular necrosis (or) fatty change. The portal tracts, hepatic sinusoids and central veins appear normal

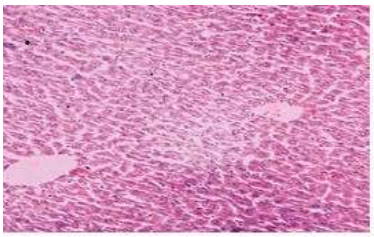

Fig. 5: Section showed that the structure liver of standard drug sylimarin. The hepatocytes in all the 3 zones appear normal. There is no evidence of hepatocellular necrosis (or) fatty change. The portal tracts, hepatic sinusoids and central veins appear normal

Figure 3 shows the histological architecture of treated liver sections with mild degree of degeneration and necrosis and indicated the moderate effect. The hepatocytes nucleases are at recovery stage and there is very minimal numbers of neutrophils. infiltration of lymphocytes and fatty changes. The combination of two extracts treated mice exhibited significant protection against PCM intoxication as evidence by the presence of normal hepatic cords and absence of necrosis with minimal inflammatory conditions around the central vein in Figure 4. In the liver section of standard drug sylimarin treated rats Normal hepatocytes and lobular structure are observed in hepatocytes. This may be due to the effective mechanisms in Figure 5. TS of liver was illustrated in Figs. (1-5).

## RESULT AND DISCUSSION

The present studies were carried out to assess the hepatoprotective activity in rats against paracetamol asa hepatotoxinn to prove its state in traditional practice against liver disorders. Paracetamol is a common hepato toxin induced hepatic injury in an experimental method for the study of hepatoprotective effects of medicinal plant extracts and drugs. Paracetamol has enhanced the levels of SGPT, SGOT, bilirubin (both total and direct bilirubin levels), alkaline phosphatase level (ALP) [27]. The results indicated that the MEETC&EECS significantly reduced the elevated levels of SGPT, SGOT, ALP and bilirubin when compared to paracetamol treated group. The MEETC&EECS has reduced the increased SGPT level from 419.65 U/L to 195. U/L, SGOT level from 282.58U/L to 79.5 U/L, ALP level from 436.51 U/L to 198.5U/L and bilirubin level from 1.89mg/dl to 0.35mg/dl.

Hepatic cells take part in metabolic activities and contain host of enzymes. In tissue, SGOT and SGPT stay alive in mitochondria. In liver injury, transport function of the hepatocytes gets troubled, resulting in the leakage of plasma membrane and thus causing an increased enzyme level in serum. The elevated activities of these enzymes are diagnostic of cellular leakage and the functional integrity of the cell membranes in the liver. ALP is excreted by the liver via bile in the liver injury due to hepatotoxins, which results in a defective excretion of bile from the liver and is reflected in their increased levels in serum. In drug-induced liver toxicity, the level of total and direct bilirubin gets elevated. MEETC&EECS marked decrease in the elevated levels of SGOT, SGPT, ALP and bilirubin (total and direct) which is nearer to the levels of control group[28]. The results are shown in Table 1.

The protective effect of the extract was exhibited at a dose level of 150 mg/kg was comparable with the standard drug silymarin. The comparison of SGOT and SGPT level were illustrated in Fig. 6.

The histological examination of the liver sections reveals that the normal liver architecture was disturbed by hepatotoxin paracetamol; rats treated with MEETC&EECS in intoxicated with paracetamol the normal cellular architecture was retained and compared with silymarin as standard, this confirmed the protective effect of the extract. In accordance with these results, it may be assumed that Triterpenoid, saponins and flavonoids, which are present in extracts, may be considered to be responsible for the hepatoprotective effect.

The MEETC & EECS have shown very significant hepatoprotection against paracetamol -induced hepatotoxicity in albino rats and has brought down the elevated level of ALP, SGOT and SGPT values than that of the drug alone and also it is comparable with standard drug.

## CONCLUSION

The MEETC & EECS have shown more hapatoprotective activity against paracetamol induced hepatotoxicity than EETC alone and it was confirmed by observing the blood reports and the Histopathology reports. In future the development of formulation by these plant constituents may give good hepatoprotective herbal medicine at lower cost.

## REFERENCES


1. Rajib Ahsan, K.M., A. Slam, Musaddik and E. Haque, 2009. Global Journal of Pharmacology, 3(3): 116-122.
2. Hazem, M. and M. Hassan, 2012. Global Journal of Biotechnology & Biochemistry, 7(2): 30-37.
3. Ward, F.M. and M.J. Daly, 1999. Hepatic Disease. In:Clinical Pharmacy and Therapeutics Churchill Livingstone, New York, pp: 195-212.
4. Vidhya Malar, H.L. and S. Mary Mettilda Bai, 2009. African Journal of Basic and Applied Sciences, 1(1-2): 21-25.
5. Sharma, S.K., 2004. Antituberculosis drugs and hepatotoxicity. Infect Genet Evol., 4: 167-170.
6. Michael, P.H. and Cynthia, 2006. Mechanisms of Drug-Induced Liver Injury. The AAPS Journal, 8(1): E48-E54.
7. Zimmerman, H.J., 1978. Drug-induced liver disease, Drugs, 16(1): 25-45.
8. Hazem, M. and M. Hassan, 2012. Global Journal of Biotechnology & Biochemistry, 7(2): 30-37.
9. Karan M., K. Vasisht and S.S Handa, 1999. Antihepatotoxic activity of Swertiachirata on carbon tetrachloride induced hepatotoxicity in rats. Phytotherapy Research, 13: 24-30.
10. Indian medicinal plants compendium of 500 species, Orient Longman Pvt. Ltd. Chennai., 2002. 320-322.
11. Nadkani, K.M., 2002. Indian material medica, Popular Prakashan, Mumbai, 2(1): 1235-1236.
12. Sisodia, S.S. and M. Bhatnagar, 2009. Hepato protective activity of *Euginajambolana* Lam. in carbon tetra chloride treated rats. Ind. J. Physiolpharmacol., 44(1): 23-27.
13. Neharkar Vishnu, S., G. Gaikwadkuldeep and B. Ghangode Deepak, 2010. Hepatoprotective activity of *Cassia alata* Linn. in rifampicin induced liver injury in rats. Advances in Pharmacology and Toxicology, 11: 27-36.
14. Syed Mansoor Ahamed, K.N. Jayaveera, Venkateswara Rao and Nagarjun Tukura, 2010. Hepatoprotective activity and Anti-oxidant activity of ethanolic extract of *Feronialimonia* leaves on paracetamol induced hepatic injury in rats. Advances in Pharmacology and Toxicology, 11: 47.
15. Vipul Gujrati, Nileshpatel, N. Venkat Rao, K. Nandakumar, T.S. Gouda, S. Shalam and M. Shantakumar, 2009. Hepato protective activity of alcoholic and aqueous extract of leaves of *TylophoraIndica* Linn in rats. Advances in Pharmacology and Toxicology, 10: 136-142.
16. Praveen, T.K., S. Dharmaraj, Jitendrabajaj, S.P. Dhanabal, S. Manimaran and M.J. Nanjan, 2009. Hepato protective activity of petroleum ether, di ethyl ether and ethanol extract of *Scopariadulcos* L. against CCl4 induced acute liver injury in mice a polyherbal formulation. Ind. J. Pharmaco, 41(3): 110-114.
17. Slater, T.F., 1984. Biochemical mechanism of liver injury. London: Academic Press, pp: 1-54.
18. Sallie, R., J.M. Tredger and R. William, 1965. Drugs and the liver. Part I. Testing liver function. Biopharm. Drug. Disp., 12: 251-259.
19. Ashok, S.K., S.N. Somayaji and K.L. Bairy, 2001. Hepatoprotective effects of *Ginkgo biloba* against carbon tetrachloride induced hepatic injury in rats. Indian J. Pharmaco, 33(2): 260-66.
20. Das, S., P. Roy, R.G. Auddy and A. Mukherjee, 2011. Silymarin nanoparticle prevents paracetamol-induced hepatotoxicity. Int. J. Nanomed, 6: 1291-1301.
21. Kotch, R.R., E.A. Glende and R.O. Rechnagel, 1974. Hepatotoxicity of bromotrichloromethane-bond dissociation energy and lipoperoxidation. Biochem. Pharmacol., 29: 1915- 2907.
22. Carvalho, M., F. Carvalho and M.L. Bastos, 2001. Is hyperthermia the triggering factor for hepatotoxicity induced by 3, 4-methylenedioxymethamphtamine An *in-vivo* study using freshly isolated mouse hepatocytes. Arch. Toxicol, 74: 789-793.
23. Hibayama, S.Y., 1992. Role of lipid peroxidation in enhancement of endotoxin hepatotoxicity. Exp. Toxicol. Patho, l44: 205-208.
24. Shtukmaster, S., P. Ljubuncic and A. Bomzon, 2010. The effect of an aqueous extract of Teucrium polium on glutathione homeostasis in-vitro: A possible mechanism of its hepatoprotectant action. Hindawi Publishing Corporatio, pp: 1-7.

25. Iriadam, M., D. Musa, H. Gumushan and F. Baba, 2006. Effects of two Turkish medicinal plants *Artemisia herba-alba* and *Teucrium polium* on blood glucose levels and other biochemical parameters in rabbits. J. Cell and Molecular Biology, 5: 19-24.
26. Jollow, D., 1973. Acetaminophen-induced hepatic necrosis. II. Role of covalent binding in vivo. Journal of Pharmacology and Experimental Therapeutics, 187: 19.
27. Kalantari, H., L.S. Khorsandi and M. Taherimobarekeh, 2007. The protective effect of the *Curcuma longa* extract on acetaminophen induced hepatotoxicity in mice. Jundishapur Journal of Natural Pharmaceutical Products, 2: 7-12.
28. Soumita Basu, Nandita Haldar, Sanjib Bhattacharya, Subhamita Biswas and Moulisha Biswas, 2012. American-Eurasian Journal of Scientific Research, 7(2): 77-81.