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Evaluation of hepato-protective activity of *Villorita cyprinoides* extract (Black water clams) against paracetamol - induced hepatic injury in albino rats

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ABSTRACT

Villorita cyprinoides is a black water clam that belongs to a group of the genus Villorita; species cyprinoides (Fam:Corbiculidae) were found in the backwaters of Kerala, mainly in Vembanad backwaters. Clams are considered to be nutritious and delicious and are fished in considerable quantities in some coastal places, known to scavenge and deactivate free radicals both in vitro and in vivo. Clams were used traditionally to treat dyspepsia, piles, general debility and some skin and lung diseases. The Villorita cyprinoides extract was investigated for its hepatoprotective effect on paracetamol (2g/kg/b.wt/p.o suspended in 0.5% CMC) induced acute liver damage in Wistar rats. Hepatoprotective activities were measured by determining the diagnostic marker enzymes such as AST, ALT, ALP, bilirubin, albumin and total protein in serum. The Villorita cyprinoides extract at the dose of 100 and 200mg/kg/p.o produced significant hepatoprotective effect by decreasing the activity of serum enzymes, bilirubin and proteins. The effects of Villorita cyprinoides extract were comparable to that of standard drug silymarin. These results suggest that Villorita cyprinoides extract have potential therapeutic value in the treatment of some liver disorders in albino (Wistar) rats.

KEYWORDS

Villorita cyprinoides(black water Clam), hepatoprotective effect, free radicals scavenger, carotenoids, paracetamol.

INTRODUCTION

Hepatic disorders, cardiovascular diseases, cancer and other chronic diseases are nowadays the most frequent causes of death. They all share a multifactorial origin and are caused by a complex interaction between genetic predisposition and personal life style[7]. For this reason an exclusively pharmacological treatment is not always sufficient and, among other factors, nutrition plays a vital contributory or protective role [10]. Liver diseases are mainly caused by toxic chemicals, excessive consumption of alcohol, infections and autoimmune disorders. Now a day's drug-induced liver toxicity is a common cause of liver injury. It accounts for approximately one-half of the cases of acute liver failure and mimics all forms of acute and chronic liver disease[8].Different types of drugs such as acetaminophen, chloroquine, rifampicin and isoniazid are inducing hepatotoxicity in the world population [15]. The rate of hepatotoxicity has been reported to be much higher in developing countries like India (8% - 30%) compared to that in advanced countries (2% - 3%) with a similar dose schedule[14].

Estuaries play a pivotal role in rural livelihood by providing valuable resources like fishes, molluscs, crabs, prawns, shrimps, *etc.* and thus constitute an important socio-economic entity. In 50 million years of evolution marine organisms anticipated many features of the modern drug industry disposable hypodermic needles, com-bination drug therapy, combinatorial strategies for drug discovery, as pharmacological agents in ion channel research and several have direct diagnostic and therapeutic potential[13]. To

prevent life-style related above diseases, it is important to rectify the poorly balanced nutritional conditions of habitual diet. These diseases are very different but share the same biochemical imbalance. Thus, carotenoids could become a new weapon to prevent and treat these diseases. Researchers are focusing on many functional ingredients in foods which may be useful for the prevention and treatment of life-style-related diseases[2]. Among them carotenoids from marine sources such as lycopene, β-carotene, lutein, zeaxanthin, tunaxanthin, astaxanthin and canthaxanthin [12, 4] were under investigation for its therapeutic and antioxidants actions. Some of the clams like *Turbinella pyrum* were used traditionally in the treatment of dyspepsia, piles, general debility, and some skin and lung diseases; *Cypraea moneta* in spleen enlargement; *Pila globosa* in sore eyes in south India; *C. Gryphoides* and *Crassostrea madrasensis were used* as demulcent[3]. One such black water clam, *Villorita cyprinoides* extract was investigated for its hepatoprotective effect on paracetamol induced acute liver damage in *rats*.

EXPERIMENTAL METHODS

Sample preparation

Villorita cyprinoides were collected from two areas of Muhamma and Nettoor in the Vembanad Lake (latitudes 9°28' and 10°10' N and longitudes 76°13' and 76°31'E in southern Kerala) of 22-20 mm size. They were immediately transported with valves shut in expanded polystyrene boxes to the laboratory. The inner muscles were removed with the aid of scissors and scalpel. The muscles were size reduced with cutter and extracted with acetone and n-hexane (1:3 ratio) and then agitated for 15 minutes by using a,magnetic agitator. The extract was filtered through cellulose under vacuum, the residue was repeatedly extracted and final extracts were made up to 3 mL. [11]. The *Villorita cyprinoides* extract **(VCE)** *was* lyophilized, transferred to amber flasks filled with N_2 and frozen (–18 °C) in sealed ampoules for further studies.

Preparation of formulation

The VCE was formulated as a fresh suspension in distilled water with 0.5%w/v CMC as a suspending agent and used for all pharmacological studies.

Experimental animal:

The institutional animal ethics committee (Register No.160/1999/CPCSEA), Annamalai University, Annamalai Nagar, India; approved the experimental design. *Albino* (Wistar) rats of 150-200g (weight) were used for the study. Animals were housed in a well ventilated room (temperature 23±2°C, humidity 65-70% and 12h light/dark cycle) at Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University. Animals were fed with standard pellet diet and water *ad libitum*. All studies were conducted in accordance with Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) norms and the National Institute of Health guidelines "Guide for the Care and use of Laboratory Animals".

Drugs and Chemicals

Silymarin, paracetamol were purchased from Micro labs Ltd., Bangalore, India. Carboxy Methyl Cellulose (CMC), was purchased from S.D. Fine Chemicals Ltd., Mumbai, India and other solvents/reagents were of analytical grade.

Paracetamol-Induced Liver Damage in Rats (Acute Model)

Five groups (I - V) each comprising of six *albino* (Wistar) female of 150-200g were selected. Group I served as control and received orally 0.5% sodium CMC (1 ml each) for seven days. Groups III & IV received oral dose of 100 and 200 mg/kg VCE respectively for 7 days. Group V received oral dose of silymarin (25mg/Kg body wt) for seven days. Paracetamol at a dose of 2gm/KG body wt p.o was administered on the 8th day to all animals in groups of II, III, IV & V.

After 48 hrs of administration of paracetamol dosing, the rats were sacrificed by cervical decapitation under Xylazine + Ketamine (16 + 100 mg/kg i.m.), blood samples were collected *via* abdominal aorta puncture, the separated serum was used for the determination of

diagnostic marker enzymes such as AST, ALT, ALP, bilirubin, albumin and total protein levels by using Secomam semi auto analyzer[16,17,6]).The results were expressed as mean ± SEM; differences in mean were estimated by means of ANOVA followed by "Dunnet's post hoc" test.

RESULTS AND DISCUSSION

Table: 1. Results of effect of VCE on biological parameters.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Bilirubin (mg/100ml)	Albumin (g %)	Total Protein (mg/dL)
Group I (Normal control)	56.30±0.315	25.21±0.496	97.10±0.194	0.89±0.008	3.79±0.14	5.24±0.035
Group II (Paracetamol control)	141.02±1.278***	64.11±2.287***	394.13±4.211***	2.17±0.025***	1.43±0.25***	3.16±0.068***
Group III (Low dose VCE group)	62.43±1.304***	28.36±0.045***	141.26±6.353***	0.94±0.04**	2.92±0.08**	4.01±0.023***
Group IV (High dose VCE group)	52.6±1.352***	22.81±0.344***	100.45±5.697***	0.91±0.008***	3.57±0.04***	5.12±0.026***
Group V (Standard Drug)	50.14±1.254***	20.73±0.547***	90.42±4.354***	0.84±0.37***	3.74±0.12***	5.10±0.045***

Values are mean ± SEM; n=6 in each group. Percentage inhibition/elevation compared to control. Group III, IV and V were compared with Group II; Group II was compared with Group I. Values are statistically significant at ** P< 0.01;* P< 0.001.**

Paracetamol is a known antipyretic, analgesic drug which produces hepatic necrosis at high doses and normally eliminated as sulfate and glucuronide conjugate. Administration of toxic doses of paracetamol the sulfation and glucuronidation routes become saturated and hence, higher percentages of paracetamol molecules are oxidized to highly reactive N-acetyl-p-benzoquinemine by cytochrome-450 enzymes. The Semiquinone radicals, obtained by one electron reduction of N-acetyl-p-benzoquineimine, can covalently binds to macromolecules of cellular membrane which increases the lipid peroxidation resulting in the tissue damage. Higher doses of paracetamol and N-acetyl-p-benzoquineimine can alkylate, oxidise intracellular GSH, results in the depletion of liver GSH pool subsequently leads to increased lipid peroxidation and thereby causes liver damage [1].

In the assessment of liver damage by paracetamol the determination of enzyme levels such as AST, ALT, ALP, bilirubin, albumin and total protein were largely used. Liver necrosis or membrane damage releases the enzyme into circulation which can be measured in serum. A high level of AST indicates liver damage, as well as cardiac infarction and muscle injury. ALT catalyses the conversion of alanine to pyruate and glutamate and is released in a similar manner. Therefore, ALT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes were indicative of cellular leakage and loss of functional integrity of cell membrane in liver. Serum ALP and bilirubin level on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure.

This present study evaluated the hepatoprotective effects of *Villorita cyprinoides* extract (VCE) in paracetamol induced liver toxicity. Acute administration of paracetamol produced significant elevation in serum - AST,

ALT, ALP & total bilirubin levels; and significant decrease in serum total protein and serum albumin level were found in toxic control group, when compared with the normal control group.

Treatment with VCE decreased the elevated serum levels of AST, ALT, ALP and total bilirubin levels towards the respective normal value and that indicates the stabilization of plasma membrane as well as repairing of hepatic tissue damage caused by paracetamol. The serum total protein and serum albumin values were almost to that of the normal values and comparable with the results observed in standard group. The above changes can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells.

Silymarin is a known hepatoprotective compound obtained from *Silybum marianum* which is reported to have a protective effect on plasma membrane of hepatocytes and possess multiple mechanisms of actions against different hepatotoxic agents. The antioxidant property and cell regenerating functions were due to the result of increased protein synthesis which were considered as most important actions[9] in the experimental animals. The above results suggest that *Villorita cyprinoides extract* (VCE) treated rats (100 and 200mg/kg) have gained normalcy against the hepatocellular injury caused by paracetamol during the 7 days treatment period and both dose levels were found to be almost equipotent.

CONCLUSION

From the results it was concluded that the *Villorita cyprinoides* extract (VCE) has significant action on paracetamol induced hepato-toxicity. Literature review as well as the preliminary chemical investigation of *Villorita cyprinoides* revealed that the *Villorita cyprinoides* extract (VCE) contains carotenoids and this may be the reason for the possible hepatoprotective activity in the experimental animals due to its antioxidant properties.

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