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Screening of Anti-ulcer activity of *Villorita cyprinoides* extract (Black water clams) against ethanol – induced ulcer in experimental 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 present study was undertaken to evaluate the anti-ulcer activity of in *Villorita cyprinoides* extract (Black water clams) against ethanol induced ulcer models in Wistar rats. The extract (100 mg/kg & 200 mg/kg) showed significant ($P<0.001$) reduction in gastric volume, free acidity and ulcer index as compared to control. This present study indicates that *Villorita cyprinoides* extract (VCE) have potential anti-ulcer activity in the ethanol induced ulcer models due to its cytoprotection activity by its antioxidant mechanism.

Keywords: *Villorita cyprinoides* (black water clam), anti-ulcer activity, free radicals scavenger, carotenoids, ethanol.

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INTRODUCTION

Peptic ulcer is a chronic, non-malignant inflammatory disease characterised by ulceration in the upper gastro-intestinal tract (stomach and duodenum) where parietal cells are found. The aetiology of gastric ulceration is multifactorial which include duration of starvation, nature of food ingested, bile reflux [9], lessened mucosal resistance[5], alteration of gastric mucosal blood flow [10], disruption of gastric mucosal barrier by stress [20], decrease in alkaline mucosal bicarbonate and mucus secretion [22], over dosage and or prolonged administration of non-steroidal anti-inflammatory drugs [21], persistent infection with *Helicobacter pylori* [15], *Zollinger-Ellison* syndrome [10], and genetic factors as suggested by a higher incidence of duodenal ulcers in patients with positive family history of this disorder or blood group type O [6]. Pathophysiology of ulcer is due to an imbalance between aggressive factors (acid, pepsin, H. *pylori* and non-steroidal anti-inflammatory agents) and local mucosal defensive factors (mucus bicarbonate, blood flow and prostaglandins). Most antiulcer drugs require prolong period of intake, yet ulcer relapse is a common occurrence [15]. Many have various adverse effects [2] and no drug proves solely effective in treating peptic ulcer. For this reason an exclusively pharmacological treatment is not always sufficient and among other factors, nutrition plays a vital contributory or protective role.

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, combination drug therapy, combinatorial strategies for drug discovery, as pharmacological agents in ion channel research and several have direct diagnostic and therapeutic potential [17]. 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. Exposure of gastric mucosa to damaging factors such as ethanol, thermal stress or various irritants that are commonly named 'breakers' of gastric mucosal barrier produces pathological changes [3]. 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 [1]. Among them carotenoids from marine sources, lycopene, β-carotene, lutein, zeaxanthin, tunaxanthin, astaxanthin and canthaxanthin [8, 14] 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([7]. One such black water clam, *Villorita cyprinoides* extract was investigated for its hepatoprotective effect on paracetamol induced acute liver damage in *rats*.

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cyprinoides extract(Black water clams) was investigated for its anti-ulcer activity against ethanol induced ulcer models in Wistar rats.

MATERIALS AND METHODS

EXPERIMENTAL METHODS

Preparation of extract

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 extracted with acetone and n-hexane (1:3 ratio) and then agitated (15 min, magnetic agitator). The extract was filtered through cellulose under vacuum, the residue was repeatedly extracted and final extracts were made up to 3 mL. (Lodeiros *et al.,* 2001) The *Villorita cyprinoides* extract **(VCE)** *was* lyophilized, transferred to amber flasks filled with N_2, and frozen (–18 °C) in sealed ampoules for the 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) male rats of 150-200g (weight) were used for the study. Animals were housed in well ventilated room (temperature 23±2^{o}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".

Ethanol induced ulcer model

The ulcer was induced by administering ethanol. All the animals were fasted for 18 hours before administration of ethanol. During this time they were kept in restraining cages to prevent coprophagy.The animals were divided into five groups, each consisting of six rats. Group I represented the control group, which received distilled water orally. Group II received ethanol, group III & IV received *Villorita cyprinoides extracts* **(VCE)** 100 and 200 mg/kg respectively, while group V (reference standard group) received omeprazole in the dose of 20

mg/kg. All the drugs were administered orally. The gastric ulcers were induced in rats by administering ethanol (90%) (1ml/200g/b.wt) orally, after 45 min of *Villorita cyprinoides extracts* **(VCE)** and Omeprazole treatment respectively. The animals were anaesthetized after 1h with Xylazine + Ketamine (16 + 100 mg/kg i.m.), and stomach was incised along the greater curvature, stretched on a piece of foam core mat, mucosal site up and ulceration was scored. [3]. Scoring of ulcer was made as follows:

Normal stomach.....(00)
Red coloration..... (0.5)
Spot ulcer..... (01)
Hemorrhagic streak...(1.5)
Ulcers.....(02)
Perforation.....(03)

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows:-

$$\% \text{ Protective} = \frac{\text{Control mean ulcer index - Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Statistics

Results of the studies were expressed as mean ± SEM; differences in means were estimated by means of ANOVA followed by Dunnet's post hoc test. Results were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Table: 1 Results of VCE treated in ethanol induced ulcer model

Groups	Ulcer Index	% Protection	pH of gastric juice
Group I (Normal control)	12.9±0.08	------	3.1±0.2
Group II (Ethanol control)	75.5±0.12***	------	2.44±0.14***
Group III (Low dose VCE group)	5.6±0.24***	54	3.5±0.12***
Group IV (High dose VCE group)	4.2±0.45***	66	3.9±0.11***
Group V (Omeprazole group)	3.5±0.07***	72	4.8±0.12***

Values are mean ± SEM; n=6 in each group. Percentage inhibition 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< 0.001.

The aetiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanisms (Piper and Stiel, 1986). To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defence mechanisms by increasing mucosal production, stabilising the surface epithelial cells or interfering with the prostaglandin synthesis. Ethanol induced gastric ulcer was employed to study the cytoprotective effect of the VCE extracts. Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intra cellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium [18, 19]. The pathogenesis of mucosal damage in the stomach includes the generation of ROS that seem to play a vital role in the formation of lipid peroxides, accompanied by impairment of anti-oxidative enzyme activity of cells [12]. VCE suppressed ulcerogenic tendencies of ethanol in the experimental rats at doses 100 and 200 mg/kg, an effect suggestive of antioxidant potential. Antioxidants disrupt the chain reaction in which free radicals turn other molecules into free radicals like themselves, a process of chain breaking or stabilization. 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 cytoprotection and antiulcer activity by its antioxidant mechanism. Further extensive studies are needed to understand the mechanism of Pharmacological actions of *Villorita cyprinoides* extract (VCE) for its cytoprotective action.

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