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Search for antihyperglycemic activity in few marine flora and fauna

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Abstract: Evaluation and identification of some new natural molecules with antidiabetic property have become one of the major preludes of present day diabetic research. Although few marine natural products are currently in the market or in the clinical trials, marine organisms still remains the greatest unexploited source of potential pharmaceuticals. Because of the unusual diversity of chemical structures isolated from marine organisms, there is intense interest in screening marine natural products for their biomedical potential. Over 300 marine organisms including flora and fauna were collected at the Institute and extracted with methanol. Their extracts were evaluated for blood glucose lowering effect. The present study reports activity profile of 30marine flora/fauna evaluated in sucrose loaded rat model. Among the marine flora , extracts of *Dolichandrone, Amoora cuculata, Chaetomorpha torta, Lumnitzera racemosa, Barringtonia racemosa* and *Excoecaria agallocha Microciona aceratoobtusa* and mollusc *Scapharca inaequivalvis* at 250 mg/kg body weight dose showed slight but insignificant lowering in the blood glucose post sucrose load in normal rats, whereas, the extract of *Cynometra ramiflora* showed significant inhibition at the same dose . Among the marine fauna the extracts of soft coral *Lobophytum pauciflorum, Sarcophyton glaucum* and the extracts of sponge *Sigmadocia pumila*, showed a little but insignificant lowering in blood glucose post sucrose load in normal rats at 250 mg/kg body weight dose.

Keywords: Antihyperglycaemic activity, marine flora, marine fauna, oral glucose tolerance test.

Introduction

There are an estimated 143 million people worldwide suffering from diabetes and this number is expected to increase to 333 million by the year 2030 (Tiwari ,2002). Therefore, the human population worldwide appears to be ion the midst of an epidemic of diabetes, can have several adverse effects. No satisfactory effective therapy is still available in modern medicine to cure diabetes. Even today, as much as ever, new treatments are needed to save and improve human lives (Ghosh, *et al*., 2001). Evaluation and identification of some new natural molecules with antidiabetic property have become one of the major objectives of present day diabetic research. Marine environment is an exceptional reservoir of biologically active products. It is one of the richest sources for floral wealth and diversity (Brad K Carte,1996) .Although few marine natural products are currently in the market or in clinical trials, marine organisms still remains the greatest unexploited source of potential pharmaceuticals. Because of the unusual diversity of chemical structures isolated from marine organisms, there is intense interest in screening marine natural products for their biomedical potential. In the light of these evidences, an attempt was made to identify some new natural antidiabetic agents using experimentally validated animal models of diabetes. For the purpose methanolic extracts of 30 marine flora (mangroves, algae and mushroom) and marine fauna (corals, sponges, shell and snail) were evaluated for blood glucose lowering effect in sucrose loaded rat model. These were the extracts of the mangroves viz. *Amoora cuculata* Roxb. (Meliaceae), *Avicennia marina* (Forsk) Vierh(Acanthaceae), *Barringtonia racemosa* Hort. Ex Miq (Lecythidaceae), *Cynometra ramiflora* L.(Leguminosa), *Dolichandrone* (Fenzl) Seem. (Bignoniaceae), *Excoecaria agollocha* L.(Euphobiaceae), *Intsia bijuga* Kuntze (Leguminosae), *Ipomoea pescaprae* L. (Convolvulaceae),*Kandelia candel* Druce (Rhizophoraceae), *Lumnitzera racemosa* Willd. (Combretaceae), *Nypa fruticans* Wurmb (Aracaceae),*Rhizophora apiculata* Blume (Rhizophoraceae), *Sonneratia griffithii* Kurz.(Sonneratiaceae), alage viz *Chaetomorpha torta* (Farlow ex F.S. Collins)(Cladophoraceae),(Halymeniaceae), *Ulva reiculata* (Ulvaceae), mushroom viz. *Ganoderma lucidum* (Ganodermaceae), sponges viz. *Dendrilla nigra* (Darwinellidae), *Sigmadocia fibulata* (Chalinidae), *Sigmadocia pumita* (Chalinidae), *Tedania anhelans* (Lieberkuhn) (Tedaniidae), corals viz. *Montipora divaricata* (Acroporidae), Green *Zoanthus* sp. (Zoanthidae), *Sarcophyton glaucum* (Alcyoniidae), *Lobophytum pauciflorum* (Alcyoniidae), shells viz. *Panaxis sucatus*

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(Planaxidae), *Thais tissoti* (Muricidae) and snail *Aplysia benedictii* (Aplysiidae).

Materials and methods

Test Samples

The marine flora/ fauna were collected from the coastal region of various states throughout the India and were identified by the Botany Division of our institute .A voucher specimen of each marine flora / fauna is deposited at the herbarium of the Central Drug Research Institute, Lucknow, India. The whole or part of the collected materials was air dried and pulverized before extraction with 95% methanol using Soxhlet apparatus at room temperature. The combined extract was concentrated under reduced pressure to either dryness or viscous mass on rotary evaporator below 40ºC.

Experimental animals

Male albino rats of Wistar strain (8 to 10 weeks of age) weighing 120 ± 20 g were procured from the animal colony of the Institute. Research on animals was conducted in accordance with the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) formed by the Government of India in 1964. The animals were housed in polypropylene cages in groups of five and the following norms were always followed for animal room light cycle; noise level 50decibel; ventilation 10-15 air changes per hour. The animal had free access to pellet diet and tap water unless stated otherwise.

Experimental design: antihyperglycaemic activity evaluation

Wistar strain albino rats of male sex were selected for this study and were kept on overnight fast of 14 to 16 hrs. Next day the blood glucose level (0 min) of each animal was measured by glucometer using glucostrips. Animals showing blood glucose level between 3.3 to 4.4 mmol/L were selected and divided into groups of five animals in each. Rats of experimental groups were given suspension of the test material made in 1.0% gum acacia at desired dose levels i.e. 250 mg/kg body weight in case of marine flora / fauna extracts and 100 mg/kg in case of standard antidiabetic drug i.e. Metformin. Animals of control group were given an equal amount of 1.0% gum acacia and the group was termed as sham control. Right after 30 min post administration of the test samples/vehicle an oral sucrose load of 10 g/kg body weight was given to the animals of all the groups. Blood glucose level of all the animals was again measured at 30, 60, 90 and 120 min post administration of sucrose load. Food but not water was withheld from the cages during the course of experimentation.

Table 1. Effect of the test samples on the blood glucose levels of the normal rats at various time intervals during the glucose tolerance test post sucrose load.

	Blood glucose profile (mM)					
Groups	0 min	30 min	60 min	90 Min	120 min	Area Under Curve
Control	3.48 ±0.33	5.84 ±0.29	6.48 ±0.20	6.62 ±0.33	6.20 ±0.35	295.8
Cynometra ramiflora	3.48 ±0.22	5.34 ±0.33	5.94 ±0.11	5.90 ±0.38	5.46 0.38±	231.9 **(-21.6%)**
Metformin	3.35 ±0.13	4.95 ±0.35	5.23 ±0.69	5.03 ±0.54	4.90 ±0.42	177.8 **(-39.8%)**

Values are expressed as mean ±S.E. of 5 animals in each group

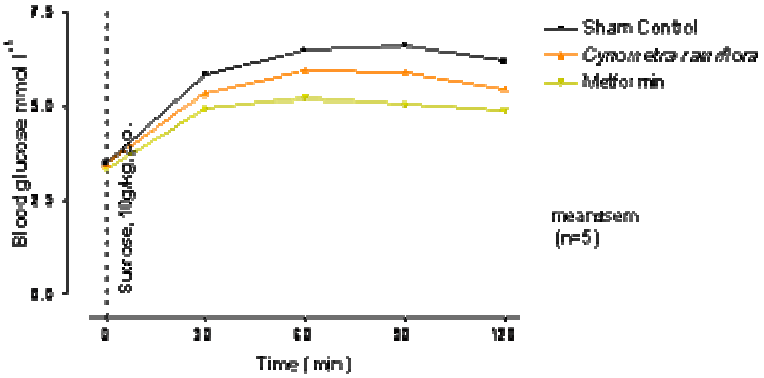
Statistical analysis

Quantitative glucose tolerance of each animal was calculated by area under curve (AUC) method using Prism Software. The area under curve of the control group and experimental group was compared to determine the percentage antihyperglycaemic activity. Statistical comparison was made by Dunnetts test. Results were expressed as mean ±S.E.

Results

The results obtained from the variance analysis showed that only one of the 30 studied extracts of marine flora/fauna declined the hyperglycaemia of the normal rats post sucrose load significantly ($p<0.05$). This extracts was of the marine flora *Cynometra ramiflora* which showed improvement of around 21.6% on glucose tolerance of sucrose loaded rats as compared to the AUC of sham control group at 250 mg/kg dose level (Table 1, Fig. 1a & 1b). Among the rest , the extracts of marine flora *Dolichandrone* showed a mild effect of around 10.2%, followed by *Barringtonia racemosa* (10.1%) , *Amoora cuculata* (10.0%), *Chaetomorpha torta* (7.75 %), *Lumnitzera racemosa* (7.07 %), *Excoecaria agallocha* (6.60 %), *Ulva reticulate*(4.15%), *Codium elongatum* (3.77%), *Ipomoea pescaprae* (3.13 %) and *Sonneratia griffithii* (2.09%) at 250 mg/kg body weight dose.The extracts of marine fauna

Fig.1 a. Acute effects of vehicle, methanolic extracts of Cynometra ramiflora (250 mg/kg body wt.) and metformin (100 mg/kg body wt.) on blood glucose during an oral glucose tolerance test.



Blood glucose values are mean ±SE of 5 animals in each group at different time interval.

Lobophytum pauciflorum showed a lowering in the hyperglycaemia to the tune of 7.21% followed by *Sigmadocia pumila* (6.87 %), *Sarcophytum glaucum* (6.44 %), *Sarcophytum palicatum* (4.70%), *Tedania anhelans*(3.84%) and *Dendrilla nigra* (3.64%).Effects of these extracts was found to be statistically insignificant as compared to the sham control group during the glucose tolerance test at 250 mg/kg body weight dose. The other studied extracts of marine flora/fauna showed no reduction in the area under the glucose tolerance curve. These extracts were of *Sigmadocia fibulata* (+8.60), *Montipora divaricata* (+5.27%), Green *Zoanthus* (+3.52%), *Planaxis sulcatus* (+8.70%),*Thais tissoti* (+2.44%), *Avicennia marina* (+8.95%), *Cauler scaleriformis* (+2.03%), *Cryptonemia undulate* (+4.90%), *Ganoderma lucidum* (+5.72%), *Instsia bijuga* (+7.44%), *Kandelia candel* (+1.89%), *Nypa fruticans* (+4.64%) and *Rhizophora apiculata* (+1.40%).

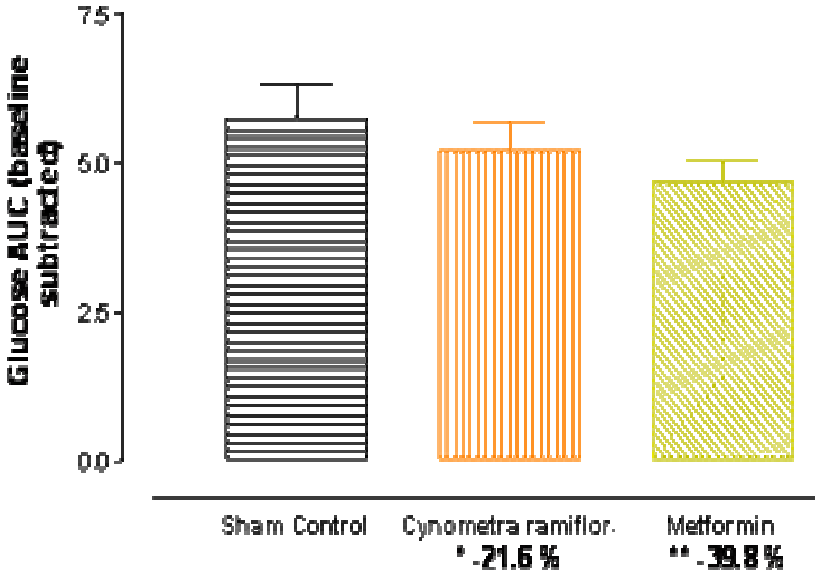
The biguanide derivative metformin was used as standard antidiabetic drug. Metformin was found to lower the hyperglycaemic peak significantly ($p<0.01$) as compared to the sham control group with 39.8 % blood glucose lowering effect.

Discussion and conclusion

An attempt was made to evaluate the collected marine flora/fauna for the antihyperglycaemic activity in sucrose loaded rats. Only one of the thirty one extracts i.e. methanolic extract of the marine flora *Cynometra ramiflora* was found to lower the postprandial blood glucose level of the sucrose loaded rats significantly. To our knowledge this is the first report describing the antihyperglycaemic potential of *Cynometra ramiflora.*

None among the rest thirty marine flora / fauna showed any promising blood glucose lowering effect in sucrose loaded rats. An extensive literature search was done on all the studied 30 marine flora / fauna but no report regarding antidiabetic activity was found in any of them. A new tetranortriterpene and eleven other compounds have been isolated from the twigs of *Amoora dasyclada* and their activity against human lung cancer cells and human liver cancer cells are documented (Yang *et al.,* 2006). Amooranin , a triterpene acid isolated from *Amoora rohituka* has potential for clinical development against human malignancies (Rabi *et al.,* 2003), but there is no report on *Amoora cuculata.* Few iridoids and flavonoids have been isolated from the mangrove *Avicennia marina* (Shaker *et al.,* 2001; Sharaf *et al*., 2000). Antinociceptive effect (Deraniyagala *et al*., 2003), antitumor property (Thomas *et al*., 2002) and antibacterial activity (Khan *et al.,* 2001) have been found in *Barringtonia*

Fig.1 b. Baseline subtracted area under the curve of vehicle, Cynometra ramiflora and Metformin treated rats post sucrose load.



*Significance: * p< 0.05, ** p< 0.01: Five animals in each group.*

racemosa . Antitumor promoting activity is reported in the diterpene isolated from the mangrove *Excoecaria agallocha* (Konoshima *et al.,* 2001), also its leaves and stem are reported to have triterpenoids and diterpinoids (Zou *et al.,* 2006; Konishi *et al.,* 2003). There is only one report regarding isolation of three fatty acid glycosides, designated pescaprsides A, B, and E from the mangrove Ipomoea pescaprae (Srivastava *et al.,* 1991). Lipid composition of the marine flora *Lunitzera racemosa* has been studied (Oku *et al.,* 2003), also the floral scent chemistry of tjis mangrove was analysed (Azuma *et al.,* 2002) but there is no report regarding any antidiabetic activity in this flora. Free radical scavenging activity is reported in the bark extract of the mangrove *Rhizophora apiculata* containing sulfated polysaccharides (Vijayavel *et al.,* 2006). Also studies were done in search of antiviral properties in this plant against human immunodeficiency virus (Premanathan *et al*., 1999). Some structural studies are reported in few species of marine alga Chaetomorpha (Shi *et al.,*2005; Rao & Ramana, 1991) but no antidiabetic activity is reported in this alga. Extract of the marine alga Ulva reticulate is reported in this alga. Extract of the marine alga *Ulva reticulata* is reported to reduce the hepatic oxidative stress in acetaminophen induced experimental rats via free radical scavenging properties (Balaji Raghavendra Rao *et al.,* 2004). Anticancer bastadin alkaloids have been isolated from a species of Dendrilla (Reddy *et al.,* 2006). There is a report regarding the growth of the encrusting sponge *Tedania anhelans* on vertical and on horizontal surfaces of temperate subtidal reefs (Knott *et al.,* 2006) but there is no report on any biological activity in this marine fauna. Few diacetylenes have been isolated from a species of the stony coral Montipora and these compounds exhibited significant cytotoxicity against a panel of human solid tumor cell lines (Alam *et al.,* 2001). Sarcophytolide , a new compound isolated from the soft coral *Sarcophyton glaucum* displayed a strong cytoprotective effect against glutamate induced neurotoxicity in primary cortical cells from rat embry (Badria *et al.,*1998). New sphingolipids and a sterol have been isolated from a species of leather coral Lobophytum of the Indian Ocean (Muralidhar *et al.,* 1998). Four new bioactive lobane diterpenes isolated from the soft coral *Lobophytum pauciflorum* were found active against the phytopathogenic fungus *Cladosporium cucumerinum*.One of the compounds was also found to be active against The Gram positive bacteria Bacillus subtilis and the yeast *Saccharomyces cerevisiae* (Edrada *et al.,* 1998). However, none of these marine flora/fauna showed any noticeable blood glucose lowering effect on glucose tolerance of the sucrose loaded rats. There are reports regarding isolation of a cytotoxic epoxy sterol from the marine mollusk *Planaxis sulcatus* (Alam *et al.,*1998). Although an antibacterial activity in the ink of the sea hare *Aplysia benedictii* is reported (Kumaran & Rajagopal, 1996) but effect of this marine fauna was found to be negligible on postprandial blood glucose of sucrose loaded rats.

In conclusion, results of the present study have uneveiled the blood glucose lowering activity in the marine flora *Cynometra ramiflora* in normoglycaemic rats. The extracts of *Cynometra ramiflora* will be further fractionated in order to search for the antidiabetic ingredients and also comprehensive pharmacological investigations are needed to elucidate the exact mechanism of the antihyperglycaemic effect of *Cynometra ramiflora.*

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