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HYPOTENSIVE ACTIVITY OF CRUDE EXTRACT OF MARINE RED ALGAE, *GRACILARIA* SP. IN RATS

W. D. RATNASOORIYA, G. A. S. PREMAKUMARA AND L. M. V. TILLEKERATNE*

*Department of Zoology and *Department of Chemistry, University of Colombo, Colombo 3, Sri Lanka*

Abstract

Hypotensive properties of the crude extract of Sri Lankan marine red algae Gracilaria sp. *of the family Gracilaracea were investigated on anaesthetized rats using two doses* (250 *or* 500*mg/kg*) *given intraperitoneally. The results show that the crude extract possesses antihypertensive properties. The extract induced an immediate fall in systolic blood pressure* (*within* 5 *min*) *which was short-lived with the lower dose and sustained with the higher dose. The precise mode of the antihypertensive action is uncertain but is likely to be mediated via decreased sympathetic activity.*

Key words : *Gracilaria* sp, red algae, hypotensive, antihypertensive, rats, crude extract, sympathetic activity.

1. Introduction

We have initiated a screening programme of Sri Lankan red algae for potential biomedicals and recently reported gastroprotection activity in a crude extract prepared from *Jania* sp (Family Corallinaceae) on ethanol-induced gastric lesions in rats (1).

In this paper we wish to report blood pressure lowering activity of a crude extract of *Gracilaria* sp. (Family Gracilaracea) one of the commonest red algae found on the rocky reefs of southern coastal waters of Sri Lanka.

2. Materials and Methods

Fresh specimens of red algae, *Gracilaria* sp. (Family Gracilaracea) were collected from the rocky reef of Beruwala on the southern coast of Sri Lanka.

The thallus of this algae (length usually between 3-9cm) has compressed axes with characteristic branching emanating from the sides of the flattened axes. A voucher specimen is deposited at the Museum of the Department of Zoology, University of Colombo, Sri Lanka (Registration No. R. A. 10).

6 Kg of this algae was air dried for 24h in shade and stored in 1:1 dichloro-methane, methanol (Petroleum Corporation, Colombo, Sri Lanka) solvent system (10L) at 30 ± 1°C. After 14 days, the solvents were decanted off and

concentrated under vacuo at 30 ± 1°C and the resultant crude (yield = 1.25g/kg) was then stored overnight under vacuo at 30 ± 1°C to remove any remaining traces of solvents.

Polyvinyl pyrrolidone (PVP) (Aldrich Chemical Co., Wisconsin, USA) coprecipitate was prepared by mixing the crude estract and PVP (extract: PVP 1:1.5w/w) in dichloromethane, methanol solvent and concentrating to dryness under vacuo at 30 ± 1°C. The coprecipitate formed was stored overnight in vacuo at 30 ± 1°C to remove traces of any remaining solvents. This was dissolved in 1ml of normal saline (0.9% NaCl) for the intraperitoneal (IP) administration to rats.

This study was carried out on randomly selected males (225-250g) from our own colony of cross-bred albino rats. These rats were anaesthetized with sodium pentobarbitone (Sagital, May and Baker Ltd., Dagenham, U.K.) given intraperitoneally (IP) (144mg/kg). A 2-3cm mid line incision was made in the neck and the right carotid artery was cannulated with a polythene catheter. The systolic blood pressure (SBP) was determined using a Condon mercury manometer (Palmer Bioscience, U.K.) at 5 min intervals upto 30 min and at 15 min. intervals over 120min following IP administration of PVP (750mg/kg) (N = 8), 250mg/kg (N = 4) or 500 mg/kg of extract (N = 8). The variation in SBP at each time point (△ SBP) is expressed in mmHg in the Figure.

To investigate the effect of the crude extract on the contractility and/or relaxability of isolated arterial vessels, rats (N = 4) were anaesthetized as described earlier. The thorax was opened and 2-4cm of the descending aorta was cut off following the application of two fine ligatures. This arterial segment was dissected free from connective tissue and was cut into two equal halves. These were mounted under 1.0g tension in a 50ml organ bath at 37 ± 1°C containing Krebs-Henseleit salt solution which was aerated continuously with 95% 0_2 and 5% CO_2 throughout the investigation period. The Krebs-Henseleit solution contained (mM) : Na^+ 14.4, K^+6.8, Mg^{++} 1.2, Ca^{++} 2.5, HCO_3^- 25, $H_2PO_4^-$ 1.2, SO_4^{--} 1.2, Cl^- 128.6 and glucose 11.1. The mounted preparations were allowed to equilibrate for 30-60min with regular washings. The circular muscle contraction or relaxations of the preparation were recored isometrically with a Washington UFI straingauges using Washington MD2 recorder.

Potassium contractions were induced by the addition of 2M KCl (to give a final bath concentration of 50mM) before and after the addition of each concentration of the extract. The extract was added sequentially with a contact period usually between 6-8min. The concentrations tested were 0.625, 1.25 or 2.5g/ml.

To investigate the effect of the extract on the urine output, 18 rats were randomly selected. These rats were then deprived of water for 12h and divided into six groups containing 3 rats each and was placed in specially constructed

metabolic cages. Each rat in three such groups were given 750mg/kg of PVP, IP, and the rats in the remaining three groups were similarly treated with 500mg/kg of extract 15min following intragastric administration of 10ml distilled water. The urine voided by these rats for a period of 6h were collected and measured.

Statistical comparisons were made with Student's "t" test and the level of significance was set at $p \leqslant 0.05$.

3. Results

The mean SBP of anaesthatised rats during the pretreatment period varied between 129.2 ± 1.53 to 144.6 ± 3.23mmHg (mean ± SEM). Intraperitonial administration of PVP (750mg/kg) did not cause any marked alteration in SBP (see Figure). In contrast, IP administration of both doses of the extract induced an immediate fall in SBP (within 5min) which lasted for about 5min. with the lower dose (250mg/kg) and about 20min with the higher dose (500mg/kg) of the extract. The percentage maximal reduction of SBP were 14.37 and 33.6 respectively with the lower and the higher dose. However, this rapid hypotensive phase was significant ($P<0.01$) only with the higher dose. With the lower dose, the SBP then recovered to normalcy, which lasted for about 45 min, and was followed by a second antihypertensive phase. However, none of these changes in SBP observed with the lower dose were significant. On the other hand, with the higher dose, the initial evanescent fall in SBP was followed by a period of sustained antihypertensive phase of lesser magnitude (16.12 — 26.34% reduction) which lasted the remaining period of study. These changes in SBP provoked by the higher dose were statistically significant (upto 60min $p<0.01$ and thereafter $p<0.05$).

In the organ bath experiment no spontaneous contractions were evident in any of the preparations used. The addition of the extract neither caused contractions nor relaxations in the isolated aortal segments. 50mM potassium elicited a sustain contraction of 0.93 ± 0.01g lasting for 6-8min. before the addition of the extract A similar contraction was also induced by potassium after the exposure of the preparations to the highest dose of the extract; magnitude 0.91 ± 0.01g; duration 6-7 min.

The mean urine output during the 6h period in the control and treated groups were 18.5 ± 3.9 and 28.5 ± 4.2ml respectively. Although, there was a 43.24% increase in urine output in the treated groups compared to the controls this effect was not statistically significant. Furthermore, during this period the treated rats showed no overt signs of toxicity or stress.

4. Discussion

In this study we investigated the hypotensive properties of the crude extract of *Gracilaria* sp, a marine red algae, belonging to family Gracilaracea, on anaesthetized rats. The results show that the extract possesses hypotensive activity. The magnitude of the antihypertensive response appeared to be dose related. In addition, the extract appears to be non toxic both *in vitro* and *in vivo.*

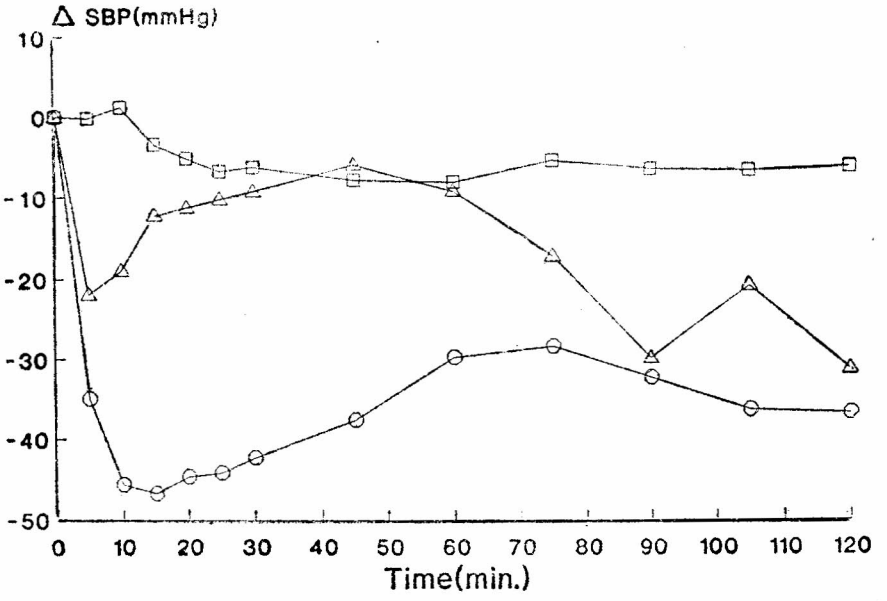

Figure : Effect of intraperitoneal administration of crude extract of *Gracilaria* sp. on maximal change in systolic arterial blood pressure (△ SBP). ⊡ —control (polyvinylpyrrolidone): △ —250mg/g extract; O--500mg/kg extract.

A low dose (250mg/kg) of extract elicited an evanescent but a transient fall in blood pressure. The high dose had a biphasic effect on blood pressure: a rapid and a pronounced reduction in blood pressure reaching a peak around 10min of administration followed by a period of sustained antihypertensive phase of lesser magnitude (about 6.21-26.34% reduction). This sustained phase lasted until the termination of the experiment (usually 2 hours). This biphasic response may indicate the involvement of at least two possible mechanisms of action in inducing the hypotensive effect. Alternatively, this could be a result of reflex trachycardia and increased cardiac output (2) or due to liberation of spasmogens such as adrenaline (3). Clarification of these possibilities however needs further investigations.

High blood pressure is of considerable concern because of the harm it can do to the heart, brain, kidneys and retina if it remains uncontrolled (2,3). The drugs currently used to alleviate hypertension act via three main mehanisms: decreased sympathetic activity; direct vasodilation; and reduction in blood volume (2.3). In this study, the onset of the hypotensive effect was quick and was not accompanied with marked diuresis. Thus, it is likely that the anti-hypertensive effect is not mediated via a reduction in blood volume. A direct vasodilatory action also seems unlikely in view of the fact that even a high dose of the extract was unable to relax the isolated descending aortal prepartion. In this respect, it is of interest to note that a Japanese commercial preparation of 'ne kombu' which is made out of basal parts of the thallus of brown algae *Laminaria japonica* is shown to have hypotensive effects due to histamines (4) and references there in). Histamines are potent vasodilators (3). Therefore, the likely mechanism of the hypotensive action of the extract investigated seems to be due to decreased sympathetic activity. Laminine, a basic amino acid isolated from a brown alga, *Laminaria augustata*, at high doses, is shown to possess hypotensive action as a result of a ganglion-blocking effect(4) It may be possible that this extract too has a similar mechanism of action. However, the precise mechanism of hypotensive action can only be ascertained by further pharmacological characterization.

In conclusion, our data show that the extract of *Gracilaria* sp, had anti-hypertensive properties. To our knowledge this is the first study to report such a bioactivity from red marine algae. Extract of another Sri Lankan red algae, *Gelidium* sp. was found to be devoid of hypotensive effects (Ratnasooriya et al., unpublished observation). Thus the hypotensive effect of *Gracilaria* appears to be species specific.

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