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Research article

## Pharmacological Activities of *Hypnea musciformis*

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**ABSTRACT:** In the present study methanol extracts of *H musciformis* were tested for their pharmacological activity on rabbit and mice. *H musciformis* significantly decreased the serum total cholesterol, triglyceride and low-density lipoprotein cholesterol levels of rabbits. This is an important finding since decreased levels of cholesterol and total lipids will minimize the incidence of many cardiovascular problems. The level of glucose is increased after the administration of *H musciformis*, which could be a transient increase only, through action on glucagon, and could also be attributed to the fact that the *H musciformis* contain many amino acids, which may form glucose. Administration of *H musciformis* significantly increased the level of dopamine. The possible effect of *H musciformis* on dopamine and other brain biogenic amines indicate that *H musciformis* probably have psychotropic and anxiolytic profile. The increased level of dopamine could also be beneficial keeping in view the etiology of Parkinsonism. In present study the level of serotonin was found to be decreased after the administration of *H musciformis*. The regular use of seaweeds as a diet will relieve the symptoms of anxiety because the known anxiolytics also manifest their effect by decreasing the concentration of serotonin.

**Key Word:** pharmacological activity, cholesterol, triglycerides, LDL, anxiety, dopamine, serotonin, Parkinsonism

### INTRODUCTION

Seaweeds have been reported to be important sources drugs and Pakistan has rich algae flora in the coastal and in shore waters of northern Arabian Sea (Hameed et al., 2000). Several studies have been reported on the photochemistry of seaweeds during last decade (Bano et al., 1994; Ara et al., 2000). The written record of the study of marine plants dates back to the third century B.C with the Greek naturalist Theophrastus, who gave descriptive account of certain copious and useful seaweeds.

There are 18 species of red seaweeds belonging to 13 families and 6 orders. Family hypneaceae contains a species *Hypnea musciformis*, whose plants are bushy,

spreading, cylindrical, 10-30 cm high, purplish green in color, cartilaginous, much branched, branches irregular, giving a bushy look to the plant (Hayee Memon and Shameel 1996). The hooked and swollen tendrils are the characteristic feature of this specie. These are also collected as drift materials. It has been reported to possess K-carrageenan (Levring et al., 1969; Andrade et al., 2000). Carrageenan is extensively used as a food additive in a wide range of products including cheese, cream, chocolate and ice creams. Its chief use is as a suspending and stabilizing agent, and has a number of pharmacological properties (Knutsen et al., 1995).

Preliminary pharmacological investigation of the algae belonging to the genus Dictyota revealed its content of considerable antibacterial, antifungal, antiviral (Nizmuddin, and Campbell, 1995), antimicrobial, antineoplastic, antifungal and cytotoxic activities (Shameel et al., 1991; Melo et al., 1997). There are reports of antispasmodic activity of *Hypnea musciformis* by Salimabi and Das (1980).

The active compounds isolated in several studies include novel mono, bi and tri cyclic diterpenoids. Among important division of seaweeds the members of

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chlorophate are less extensively investigated. The present study was designed to evaluate the pharmacological activities of *Hypnea musciformis* collected from Karachi coast.

## MATERIAL AND METHOD

### Plant material

Seaweeds were collected from Paradise Point and Manora of Karachi. After being identified by a qualified taxonomist, the collected *Hypnea musciformis* were drained of seawater and epiphytes as well as the calcareous deposits were removed. Fresh seaweeds were dried completely and then soaked in methanol (about 2 lit × 3) for thirty days. After thirty days, the extract was filtered and the residue left was the methanolic extract (Siddiqui et al., 1993), tested for various pharmacological activities.

### Animals

Rabbits (average wt = 1700g) and albino mice (average wt = 20g) maintained by commercial diets were used for the experiments. Following preliminary experiments, an optimum dose of 23.5mg/kg was arrived at for rabbits and 25mg/kg in mice. Extracts were administered orally for 14 days.

### Estimation of Hematological parameters

After 14 days of treatment, animals blood was collected from the experimental animals and analysed for the following parameters using standard laboratory techniques: Red Blood Cell (RBC) count, Hemoglobin and Erythrocyte Sedimentation Rate (ESR). Values for Colour Index (CI) and Corpuscular Hemoglobin content were derived from the values of RBC count and Hemoglobin concentration:

### Estimation of Biogenic amines

Animals were decapitated and thereafter the brain was taken out and desired brain sections were identified with the help of stereotaxic atlas (Paxinos and Watson, 1982). Homogenates were then prepared from the tissue samples collected. Estimation of monoamines and their metabolites was carried out as described by Haleem et al. (1988) using high performance liquid chromatography with electron capture detection (HPLC-EC). EC detection was accomplished using L-ECD-6A EC detector (Shimadzu) with glossy carbon electrode. The potential utilized was +0.8 to ±1.0 V vs Ag/AgCl as a reference electrode.

### Effects of Seaweeds on neuropharmacological activities in mice

The effects of seaweeds on a number of neuropharmacological activities were assessed using standard techniques as described below:

*Open field activity:* The procedure used was essentially as described earlier by Haleem et al. (1988). The activities were scored by counting squares crossed by each mouse during a 5-minute period.

*Rota-rod test:* Mice were placed on horizontal rod, rotating at 16 rpm. Animals, which remained on rod for 120 seconds were selected for further studies; such animals were placed on rod at different time intervals. The time of the fall from the rod was noted (Dienham and Miya, 1957).

*Hypogenic activity (righting reflex):* Mice were placed gently on their back on an iron surface at 30 degrees. Loss of righting reflex was said to have occurred if mouse remained on its back for more than 30 seconds. Sleeping time was measured as the time elapsed between the losses and regaining of the righting reflex.

*Exploratory activity (head-dip test):* This consisted of placing female mice 45 minutes after giving extract and control, simply on wooden board with 10 evenly spaced holes and counting the number of times the dipping of the head in the holes during 5 minutes trial.

*Stationary rod:* Prior to treatment, mice were given a brief training period (2 or 3 trials), to ensure their ability to walk across a horizontal steel rod. The mice are placed individually on the mid-point of the rod and forced to walk towards a platform at either end of the rod.

*Swimming-induced depression:* The mice were allowed to swim in a water bath till the mice showed the signs of despair. The same procedure was adopted for control and treated animals.

### Biochemical analysis

*Estimation of Total Protein and Total Lipids:* Total protein was estimated by direct Biuret method (Peters, 1968).

*Estimation of Blood Glucose:* Blood glucose was estimated using the O-toluidine method (Dubowski, 1962).

*Estimation of Total Cholesterol:* Total cholesterol was estimated by enzymatic colorimetric method (Allain et al, 1974; Meattini et al, 1978).

*Estimation of Triglyceride:* Estimation of triglyceride was done by enzymatic colorimetric method (Rietz and Guilbault, 1977).

**Statistical analysis:**

One-way ANOVA, t- test, and Newman-keuls tests were employed in the analysis of the neurochemical and biochemical data.

**RESULTS**

The effect of chronic administration of *H musciformis* on biochemical profile of rabbit blood is shown in Table 1. The Table shows significant increase in total proteins (p<0.01) and blood glucose (p<0.01) levels but serum triglycerides (p<0.01), cholesterol (p<0.01) and lipids (p<0.01) were reduced significantly.

**Table 1.**

The effects of *H musciformis* on biochemical profile of rabbit blood.

	Control n=6	<i>H musciformis</i> n=6	t-test
<b>Total Proteins</b> (mg/dl)	7.3±0.1	*10.1±0.6	t=9.5 p<0.01
<b>Triglycerides</b> (mg/dl)	365±8.5	*120.4±13.3	t=41 p<0.01
<b>Glucose</b> (mg/dl)	113±1.2	*119.9±1.9	t=12.9 p<0.01
<b>Cholesterol</b> (mg/dl)	230.8±7.7	*161.6±5.4	t=19.7 p<0.01
<b>Lipids</b> (mg/dl)	683±5.4	*530±9.4	t=32.6 p<0.01

Values are means ± SD 14 days after chronic administration  
n= total number of animals per group

Significant difference by t-test

\*p < 0.01 from saline injected control following t-test

**Table 2**

Effect of *Hypnea musciformis* on the Hematological profile of mice blood

	Control n=6	<i>H musciformis</i> n=6	t-test
<b>Hemoglobin</b> g/dl	13.0±0.7	12.5±1.3	t=0.7 IS
<b>Erythrocyte Count</b> million/mm <sup>3</sup>	4.8±0.1	4.7±0.5	t=0.3 IS
<b>Erythrocyte Sedimentation Rate</b> mm/hr	2.1±0.1	2.0±0.2	t=0.2 IS
<b>Hemoglobin Content</b> g/dl	26.1±1.4	27.9±5.8	t=0.6 IS
<b>Color Index</b>	8.2±0.9	7.8±0.8	t=0.8 IS

Values are means ± SD 14 days after chronic administration  
n= total number of animals per group

IS= Insignificant difference by t-test

The effects of chronic administration of *H. musciformis* on direct and derived hematological parameters in rabbits are presented in Table 2. No significant changes were observed in the values of hemoglobin, erythrocyte count, ESR, hemoglobin content and color index when compared with the control.

**Table 3**

Effect of *H. Musciformis*. on the Brain Catecholamines and Indolamines

	Control n=6	<i>H musciformis</i> n=6	t-test
<b>Catecholamines</b>			
<b>DA</b> ng/g	167.0±17.7	290.5±40.1	t=6.3 p<0.01
<b>DOPAC</b> ng/g	156.9±19.7	77.2±13.9	t=9.5 p<0.01
<b>HVA</b> ng/g	307.0±27.5	156.2±8.4	t=15.3 p<0.01
<b>Indolamines</b>			
<b>5-HT</b> ng/g	148.9±16.3	117.3±12.4	t=3.7 p<0.01
<b>5-HIAA</b> ng/g	147.4±20.9	84.8±7.4	t=6.3 p<0.01

Values are means ± SD 14 days after chronic administration  
n= total number of animals per group

Significant difference by t-test

\*p < 0.01 from saline injected control following t-test

**Table 4.**

Effect of *H.Musciformis* on activity

	Control n=6	<b>H musciformis</b> n=6	t-test
<b>Open field</b> crosses	106±7.6	80±8.4	6.1 p<0.01
<b>Stationary rod</b> time in sec	5.9±0.6	9.5±1.5	5.4 p<0.01
<b>Head dip</b> no	43.5±3.2	33.0±3.5	9.1 p<0.01
<b>Rota rod test</b> 16 rpm	121.5±3.2	110.1±7.2	4.6 p<0.01
<b>Righting Reflex</b> time in sec	1.5±0.5	2.1±0.4	3.1 IS

Values are means ± SD 14 days after chronic administration  
n= total number of animals per group

Significant difference by t-test

\*p < 0.01 from saline injected control following t-test

The effect of chronic administration of *H musciformis* on neurochemistry of mouse brain is presented in Table 3. The table shows significant increase in the level of DA (p<0.01) and decrease in the

levels of DOPAC ( $p < 0.01$ ), and HVA ( $p < 0.01$ ), 5-HT ( $p < 0.01$ ) and 5-HIAA ( $p < 0.01$ ) were also reduced significantly.

The neuropharmacological activities of the control and experimental animals are presented in Table 4. The activity of mice administered *H musciformis* showed significant decrease in open field ( $p < 0.01$ ), head dips ( $p < 0.01$ ), and rota rod ( $p < 0.01$ ), whereas it increased the stationary rod activity significantly ( $p < 0.01$ ). There was however no significant change in the righting reflex.

## DISCUSSION

In the present study, the metabolic and pharmacological effects of a common species of seaweed, *H musciformis* was investigated. Blood lipids, cholesterol and triglycerides were shown to be decreased after the administration of *H musciformis*. This is an important finding since decreased levels of cholesterol and total lipids minimize the incidence of many cardiovascular problems. (Bersot *et al.*, 2003). The use of seaweeds in Asian diet may be responsible for the relatively normal ranges of lipid profile in this region (Salimabi and Das, 1980). Literature survey indicates that seaweeds contain unsaturated fatty acids more than saturated fatty acid, the main fatty acid being cholesterol and many other sterols (Aliya *et al.*, 1991). Ingestion of diets containing highly unsaturated fatty acids has been shown to depress blood cholesterol level (Grundy, 2004). Ahmed *et al.*, 1993 also reported the anti-hypertensive effect of seaweeds.

The increase in the level of dopamine after the administration of *H musciformis* suggests that activity can be increased and may relieve signs and symptoms of depression. This suggestion is based on the fact that the reduction in the central dopamine concentration can lead to depression and drugs which can increase the central dopamine concentration reverse depression (Sadock, 2000). Recent observations also support dopamine as an important factor in depression (Ghaemi *et al.*, 2004). The increase in the level of dopamine could also be beneficial in Parkinsonism. The findings of the present work on dopamine and its metabolite DOPAC, and HVA indicate that the levels of dopamine increased but the level of its metabolites was decreased indicating slow metabolism of dopamine. This slow metabolism can also increase the level of dopamine (Albin and Frey, 2003), which could be beneficial in Parkinsonism. This work could be further extended to confirm the areas of brain where dopamine was increased, and can be explored for its use in cognitive disorders (Albin. *et al.*, 1989).

Serotonin (5-HT) is another neurotransmitter involved in the depression and anxiety states (Bluer *et al.*, 1990). Serotonin acts as an inhibitor of pain pathways in the spinal cord and its action in the higher region of the nervous system is believed to help control the mood perhaps even to effect the sleep cycle (Glenon, 1990; Olivier *et al.*, 1991). The norepinephrine and serotonin system normally provide drive to the limbic system, but excess can cause mania (Mongeau *et al.*, 1997). The pleasure and reward centers of the hypothalamus and surrounding areas receive large numbers of nerve endings from norepinephrine and serotonin systems (Tork, 1990). In the present study the level of 5-HT was found to be decreased after the administration of *H musciformis*. The possibility of this seaweed species having anxiolytic properties may not be ruled out since known anxiolytics are known to produce their effect by decreasing the concentrations of 5-HT (Gray and McNaughton, 2000; Cervo *et al.*, 2000).

In the present work, Open field activity was reduced significantly. Also, the righting reflex was positive as CNS depressant effect was not marked. The affect on the Rota-rod test showed that the animals that were previously trained on 16 rpm and were not falling, after the administration of the extracts the animal showed decreased muscular grip and resulted in falling. The anxiolytic activity due to serotonin may have contributed to these observations as the mice became relaxed and did not try to balance. (Gray and McNaughton, 2000).

In conclusion, the study underscores the therapeutic potentials of the common seaweed, *H- musciformis*. Further work is needed on other seaweeds before a sweeping generalization may be made. It will also be necessary to assess the therapeutic properties at different times of the year, since the chemical composition may vary with season.

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