

Full Length Research Paper

Some biological studies on *Hypnea pannosa* J. Ag.

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The present investigation focused on studying the toxicity, analgesic, behavioral and anti-emetic activities of the ethanol extract of *Hypnea pannosa*. The ethanol extract caused 100% lethality at the doses of 250 and 500 mg/kg. Significant analgesic and central depressant effects were observed from 150 mg/kg dose. The extract at 200 mg/kg dose exhibited significant anti-emetic effect, when compared to standard drug.

Key words: *Hypnea pannosa*, toxicity, analgesic, behavioral, anti-emetic activities, crude extract.

INTRODUCTION

The utilization of marine algae for medicinal purposes is not new; however, the discoveries of the last few years have opened new and important possibilities in the field. The earliest particulars on sea weed utilization originate from Chinese *Materia Medica* of Shen-nung written in 2700 B.C. Dioscorides (40-90 AD) mentioned the use of algae in medicines of earlier civilization and also indicated their importance during these times (Schwimmer et al., 1955). The Chinese, Japanese, Philippines, and other South and East Asians used algae not only as food, but also as medicine. Long before scientific research began, numerous algae were used as medicaments, especially in coastal countries. They were used in folk medicine against goiter, nephritic disease, worms, catarrh etc. In different pharmacopoeias and pharmaceutical handbooks, a number of algae are mentioned as medicinal agents. Many of their constituents have pharmaceutical and medicinal value. They are used as medicaments and auxiliaries (Zaneveld, 1959).

The present study on *H. pannosa* was undertaken to screen this marine red alga for its biological activities. *H. pannosa* belongs to the family Rhodophyceae. It grows as cushions of interwoven filaments attached to rocks near the lower mark of the marine littoral zone at Buleji,

Karachi.

The genus is represented by three species at northern sea coast of Pakistan; *H. pannosa* is one of them. The *Hypnea* species have often been subjected to phytochemical and biological investigations. The literature survey indicated that this genus contained sterols, fatty acids, carbohydrates, terpenes, proteins etc (Tsuda et al., 1959; Fattorusso et al., 1975; Cambaut et al., 1984; Kato et al., 1982; Mahran et al., 1985; Hussain et al., 1991; Bruni et al., 1974). Studies on *Hypnea musciformis* suggest that it possess anti-inflammatory and antifungal activities (Naik et al., 1980; Melo et al., 1997).

MATERIALS AND METHODS

Plant material

The screening of the plant was carried out using crude extract of the red alga. The plant material, *Hypnea pannosa* was collected from coastal area near Karachi and dried under shade.

Preparation of the extract

The dried alga was crushed and soaked in ethanol. The extract was removed after ten days and evaporated under reduced pressure.

Animals

In the present study, Swiss albino male mice weighing 20 to 25 g,

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male Wistar rats weighing 180 to 200 g and young male chick, 4 days old weighing from 32-52 g were used as test animals. Permission and approval for animal studies were obtained from Board of Advanced Studies and Research, University of Karachi as per the recommendation set and approved by Helsinki (1996) for animal handling and care.

Toxicity assessment

The toxicity assessment of *H. pannosa* was carried out using male Swiss albino mice. The animals weighing 20-25 g were divided into two groups, the extract treated "Test-group" and DMSO treated "Control-group". Groups were made three days prior to the study, each having six animals. The animals were maintained under laboratory conditions of temperature $23\pm 3^{\circ}\text{C}$ with 12 h dark and 12 h light cycles. However, they had free access to food and water. All efforts were made to minimize animal suffering and reduce the number of animals used.

The crude ethanol extract of *H. pannosa* was administered by intra-peritoneal (i.p.) route to the test group at the doses of 150, 250 and 500 mg/kg body weight.

Mice in the control group only received vehicle. Both groups had free access to food and water. The animals were observed for any abnormal behavior, such as sedation, motor impairment and hyper excitability for 3 h. Further, the incidence of mortality was noted up to 24 h after administration. The number of deaths from plant extract was recorded within this period of time (Sayyah et al., 2004; Moreira et al., 2003; Young et al., 2005).

Analgesic activity

The purpose of the study was to evaluate the anti-nociceptive activity of *H. pannosa* using classical pain models in mice. For the study, Swiss albino mice were used. The analgesic activity was carried out by observing the reaction of mice to the thermal stimulation of the tail tip on immersion in water, maintained at 52°C . The reaction time was noted 30 min before and 30, 60, 90, 120, 150 and 180 min after treatment. The mice were divided into three groups, each having six animals. The groups were administered DMSO, Pethidine (50 mg/kg), and ethanol extract of *H. pannosa* (150mg/kg) i.p (Matos et al., 2004). DMSO and Pethidine served as controls.

Baseline latency (reaction time) was obtained with three measurements, after each measurement, a cut off time of 20 s was used to prevent tissue damage. The mean of these three measurements is the pre-drug latency time. Readings were taken at the given intervals and after drug administration, mean of these three readings were considered as the post drug reaction time. Tail flick latency difference (TFLD) or mean increase in latency after administration was used to measure the analgesia produced by test and standard drugs. TFLD was calculated as:

Analgesia TFLD= (Post drug TFL- Pre drug TFL)

The values were expressed as mean \pm SEM. Statistical significance was determined using the student's t-test. Values of $p\leq 0.50$ and 0.01 were taken to imply statistically significant and highly significant, respectively.

Behavioral activity

The behavioral activity of the animal was studied using open field method. Male Wistar rats weighing 180-200 g were divided into drug treated "test" and DMSO treated "Control" groups of six animals each. Groups were made three days prior to the study

each. The animals were maintained under laboratory conditions of temperature $23\pm 3^{\circ}\text{C}$ with 12 h dark and 12 h light cycles. They had free access to food and water. All efforts were made to minimize animal suffering and reduce the number of animals used.

The crude ethanol extract of *H. pannosa* was dissolved in DMSO and administered i.p. to the test group at the dose of 75 mg/kg according to their body weight. Control group only received DMSO by the same route.

The open field apparatus consisted of square area 76×76 cm, with 42 cm high walls. Floor of the apparatus was divided by lines into 25 equal squares. The rats were exposed to the open field after 30 min of receiving drug.

The number of crossings (locomotion), number of rearing and the total immobility time were recorded for 5 min. The open-field apparatus was then cleansed with 5% ethanol before introducing the next animal in order to preclude the possible clueing effects of odors left by previous subjects. To minimize possible influence of circadian changes on the rat open-field behavior, control and experimental animals were intermixed (Shahidi et al., 2000).

The values were expressed as mean \pm SEM. Statistical significance was determined using the student's t-test. Values of $p\leq 0.50$ and 0.01 were taken to imply statistically significant and highly significant responses, respectively.

Anti-emetic activity

In this study, the potential of anti-emetic activity of ethanol extract of *H. pannosa* was determined. Effects produced by principles present in the extract are determined by decrease in the number of retchings after oral administration of Copper sulfate. Young male chicks, 4 days old weighing 32 to 52 g, were used as test animals. The anti-emetic activity was determined by calculating the mean decrease in number of retchings in contrast with those of the control (Yang et al., 1999). Animals were divided into 4 groups, each having six animals. The animals were set aside individually in a large beaker to stabilize for 10 min. The extract of *H. pannosa* was dissolved in 0.9% saline containing 5% DMSO and 1% Tween 80 and administered abdominally at a volume of 10 ml/kg of the body weight of the animals. The test and standard drug (Chlorpromazine) were administered at 200 mg/kg abdominally. Control group received only 0.9% saline. After 10 min, Copper sulfate was administered orally at 50 mg/kg and the numbers of retching (an emetic action without emitting gastric material) was observed after 10 min. The criteria for anti-emetic effect is to observe the decrease in numbers of retching in contrast with those of control. The percent inhibition was calculated by the following formula:

% Inhibition = $\frac{A-B}{A} \times 100$

Where, A is the frequency of retching in control group; B is the frequency of retching after sample treatment in test group. The value for anti-emetic activity was expressed as mean \pm SEM. The statistical significance of the difference was determined by an unpaired student's t-test.

RESULTS AND DISCUSSION

Toxicity assessment

The toxicity assessment of the crude ethanol extract of *H. pannosa* (Table 1) at the dose of 150 mg/kg did not show any toxic signs, side effects, behavioral change or lethality in animals. It indicates that the extract is well tolerated and safe at this dose. However, the same

Table 1. Toxicity assessment of *Hypnea pannosa*.

Treatment (i.p)	Incidence of mortality	Percentage mortality
Control	0/6	0
150 mg/kg	0/6	0
250 mg/kg	6/6	100
500 mg/kg	6/6	100

N=6.

Table 2. Analgesic effects of *Hypnea pannosa*.

Treatment (i.p)	Dose (mg/kg)	Analgesia TFLD (mean increase in latency after drug administration \pm SEM)					
		30 (min)	60 (min)	90 (min)	120 (min)	150 (min)	180 (min)
Drug	150	1.667 \pm 0.68	25.0 \pm 0.6	29.006 \pm 0.683	30.17 \pm 0.70	30.36 \pm 0.68	27.22 \pm 0.71
Control (DMSO)	150	1.378 \pm 0.40	1.33 \pm 0.3	1.25 \pm 0.297	1.317 \pm 0.35	1.289 \pm 0.30	1.44 \pm 0.45
Pethidine HCl	50	2.26* \pm 0.63	3.52** \pm 0.46	2.82** \pm 0.13	2.57** \pm 0.23	1.92** \pm 0.07	1.57* \pm 0.20

N=6; * P<0.05; ** P< 0.01.

Table 3. Effects of *Hypnea pannosa* extract on the behavior of rats in open field test.

Compound	Dose in (mg/kg)	Number of squares crossed in 5 min
Control	-	156.83 \pm 3.67
Extract of <i>Hypnea pannosa</i>	150	128.33 \pm 6.32

N=6.

extract at the dose of 250 and 500mg/kg produced toxic side-effects. The animals exhibited decreased spontaneous motor activity, loss of sensation, paralytic effect and died within 30 min of drug administration. However, the exact mechanism on how the extract works is not known.

Analgesic activity

The analgesic effect of *H. pannosa* given in Table 2, shows that the tail flick latency times at 90, 120 and 150 min are highly significant at a dose of 150 mg/kg. This significant increase in the reaction time for tail flick method indicated the analgesic effect of *H. pannosa* extract and also elucidates the involvement of central mechanism in analgesic action. Analgesic effect mediated through central mechanism indicates the involvement of endogenous opioid peptides and biogenic amines, such as 5HT (Bensemana and Gascon, 1978; Glazer et al., 1981). From chemical point of view, *H. pannosa* contains fatty acids, sesquiterpenes and sterols (Afaq et al., 1992), therefore it may be inferred that the ethanol extract of *H. pannosa* showed analgesic activity due to the presence of these compounds.

A comparison of analgesic effect of *H. pannosa* with those of other medicinal plants shows that it has more potent effect than others (Stasi et al., 1988). *Arnica*

montana is used for relieving pain from bruises, sprains, tendon dislocation and increasing the re-absorption of internal bleeding in dose of 200 mg/kg. *Artemisia absinthium* has a marked tonic effect and used to relax muscles and treat rheumatism, it is recommended in a dose of 180 mg/kg. Likewise, 250 mg/kg of *Ocimum sanctum* reduces joint pain and blood sugar levels. *Cinnamomum camphora* is used to relieve back ache, arthritic and rheumatic pain at a dose of 200 mg/kg. *Apium graveolens* is recommended at dose of 190 mg/kg to treat rheumatism (Jones et al., 2005). Therefore, it is concluded that the crude extract of *H. pannosa* exert more profound and sustained analgesic effect than other natural products, at a much lower dose of 150 mg/kg.

Effects of *H. pannosa* on the behavior of rats in open field test

To study the locomotion and exploratory behavior of animals, different approaches like latency to move and the number of squares crossed are used. Both are pharmacologically and behaviorally valid methods.

The present investigation on behavioral changes in rats was carried out using open field method. This method is used for the measurement of behavioral activity of small animals. The results observed in the open field model (Table 3) showed significant differences between the

Table 4. Anti-emetic effects of *Hypnea pannosa* extract.

Drug	Dose	Number of animals/group	Mean number of retches±SEM	Percentage Inhibition
Control	-	06	69.28±4.2	-
Chlorpromazine	200	06	46.42±3.84*	32.99
<i>Hypnea pannosa</i> extract	200	06	41.3±2.10*	40.38

N=6; * P<0.05.

control and experimental groups. Locomotion frequency, rearing frequency and immobility duration in the open field were altered after the administration of extract. The decrease in the spontaneous motor activity indicated that the extract has central depressant action. The administration of the crude extract of *H. pannosa* produced a reduction in locomotor activity.

The mechanism of this depression is not clearly understood at this point, but it can be assumed that the ethanol extract of *H. pannosa* may exert CNS depressant effect by interfering with the function of cortex.

Anti-emetic activity

The anti-emetic effects of the ethanol extract of *H. pannosa* are shown in Table 4. It shows that the extract of *H. pannosa* inhibited emesis to a greater extent than chlorpromazine at 200 mg/kg. *H. pannosa* extract showed 40.38% inhibition compared to chlorpromazine, which showed 32.99% inhibition at P<0.05. Although, the results are significant and comparable to that of chlorpromazine, but the mode of action is not known. However, as the oral copper sulphate induces emesis by peripheral action (Hossein et al., 2005) and the peripheral 5-HT₄ plays an important role in this action (Bhandari and Andrews, 1991; Fukui et al., 1994). The extract of *H. pannosa* was able to effectively prevent its effect, it could be implied that *H. pannosa* extract has a peripheral anti-emetic activity. Further studies are required regarding the actual mechanism of action and the active compounds responsible for anti-emetic activity of *H. pannosa* extract.

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