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Pharmacological Studies on Hypnea musciformis (Wulfen) Lamouroux

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Abstract: Hypnea musciformis belonging to family Rhodophyceae Genus name is Hypnea. To the best of our knowledge the algae Hypnea musciformis was evaluated for Phytochemical study Such as Physico-chemical analysis, elemental study, metal analysis. The different extracts undergo Preliminary Phytochemical analysis for the identification of various Phytoconstituents. It answers positively alkaloid, carbohydrate, glycosides, tannins, protein, amino acid and steroid ...Pharmacological activity was screened by which methanol extract showed the maximum inhibition of arthritis. Then Methanolic extract was subjected to column chromatography to isolate the compound and identified by TLC and confirmed as Flavonoid by spectral studies as Astaxanthin and Hesperidin. Which responsible for reduction of arthritic activity and Free radical like Nitric oxide and DPPH. In Histopathological studies Methanolic extract of Hypnea musciformis shows effective in curing the synovial damage as compared to arthritic control. Our result showed that the methanol extracts and isolated compound possess significant anti-rheumatoid activity. It may due to the presence of Phenolic and Carotenoids terpene constituents. From the above results it can be concluded that Hypnea musciformis can be used in the treatment of anti-rheumatoid arthritic disease as a novel drug on the basis of clinical trials. Chemistry of marine natural products is a newer area of potential resources for discovering new therapeutic tangents developing new leads.

Key words: Hypnea musciformis, Methanolic extract, Antirheumatoid activity, Antioxidant activity, Phytoconstituents

SUMMARY

Aim and Objective: An algae plays a vital role in the management of various chronic diseases. According to Literature review *Hypnea musciformis* red algae was found to be used for Antioedema , anti-inflammatory, Antispasmodic, Antiviral, Anti bacterial, Antifungal, chronic bronchitis . From the detailed review of literature which afforded no information on the Anti rheumatoid potential of the algae. Thus algae of *Hypnea musciformis* red algae were selected for the present work.

Materials and Methods: The algae specimen for the proposed study were collected from the Coastal area of Tamil Nadu in Mandapam-District-Rameswaram during the month of January 2010 and it was identified and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Center, (PARC) Tambaram, Chennai where the voucher specimen was deposited (no. 512) in the Pharmacognosy herbarium, Vels University, India.

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ETHNOBOTANICAL PREFACE (*Guis et al.,* (1779)

***Hypnea musciformis* (Wulfen) Lamouroux [1]**

Division	:	Rhodophyta
Order	:	Gigartinales
Family	:	Hypneaceae
Genus	:	Hypnea
Species	:	corticata
Taxonomic name	:	*Hypnea musciformis (Wulfen) Lamouroux*
Synonyms	:	*Fucus musciformis (Wulfen 1791), Hypnea rissoana*
Common names	:	*Hypnea*
organism type	:	Algae .*Hypnea musciformis* (basionym focus musciformis) is **Classified as red algae.**

PHYSICOCHEMICAL ANALYSIS OF *HYPNEA MUSCIFORMIS*

ASH VALUES

Determination of total ash: 2 gm of air-dried crude drug was weighed accurately in a tarred platinum or silica dish and was incinerated at a temperature not exceeding 450°C until free from carbon. It was then cooled and weighed. The percentage of ash was calculated with reference to the air-dried drug.

Determination of Water-soluble ash:
The total ash was boiled for 5 min. with 25ml of water. The insoluble matter was collected in a gooch crucible or an ash less filter paper. It was washed with hot water and ignited for 15 min at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash; the Differences in the weight of the ash represent the water-soluble ash. The percentage of Water-soluble ash was calculated with reference to the air-dried drug.

Determination of Acid insoluble ash: The total ash was boiled with 25 ml of 2M Hydrochloric acid for 15 min. The insoluble matter was collected in a gooch crucible or an ash less filter paper. It was washed with hot water and ignited. It was then cooled in Desiccators and weighed. The percentage of acid insoluble ash was calculated with reference to air-dried drug.

Determination of Sulphated ash: 2 g of powdered drug was taken in an accurately weighed silica crucible, ignited gently at first until the substance is thoroughly charred and cooled. The residue moistened with 1 ml of dilute sulphuric acid, gently heated until White fumes was no longer evolved and ignited at 800°C $\pm$ 25°C until black particles have disappeared. Crucible allowed for cooling, few drops of dilute sulphuric acid added and heated. Ignited as before, allowed to cool and weighed. The percentage of sulphated ash Was calculated with reference to air-dried drug.

EXTRACTIVE VALUES

Determination of Alcohol soluble extractive:
5 grams of the powder was macerated with 100 ml of alcohol of the specified strength in a closed flask for 24 hours, shaking up frequently for 6 hours and allowing standing for 18 hours. It was filtered rapidly taking Precautions against loss of alcohol and 25ml of the filtrate was evaporated to dryness at 105°C and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.

Determination of chloroform soluble extractive

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5 grams of the powder was macerated with 100ml chloroform in a closed flask for 24 hours, shaking frequently for 6 hours and allowing standing for 18 hours. It was filtered rapidly taking precautions against loss of alcohol and 25ml of the filtrate was evaporated to dryness at 105°C and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug.

DETERMINATION OF LOSS ON DRYING:

A weighed stopper glass bottle dried for 30 minutes under the same conditions to be employed in the determination of the weighed sample. The sample was placed in to the bottle and the contents were accurately weighed. The sample was distributed evenly to depth no exceeding 10 mm. The loaded bottle was placed in a drying chamber (oven) and the stopper was removed. The sample was dried to constant weight at a temperature of 110° C in hot air oven. The percentage of loss on drying was calculated with reference to the air-dried drug. All reports was tabulated in **table: 1**

PHYTOCHEMICAL STUDY OF *Hypnea musciformis*

INORGANIC ELEMENTAL ANALYSIS OF *Hypnea musciformis*

Procedure: Ash of powder was prepared and 50% v/v hydrochloric acid was added to ash, kept for 1 h or longer and filtered. The filtrate was used to determine the presence or absence of calcium, carbonate, chloride, iron, magnesium, nitrates, phosphate, potassium, sodium and sulphate by means of various chemical tests. The findings are mentioned in **(Table no.2)**

METAL ANALYSIS OF *Hypnea musciformis*

Instrument Used in the Metal Analysis

All metals are analyzed by Atomic Absorption Spectrophotometer

Model No. : Vs 00141

Year of make: 2004

Lamps used for analysis: Varian

Metal content of *Hypnea musciformis* determined by using Atomic Absorption Spectrophotometer meter and followed the standard procedure**. (Table no.3)**

EXTRACTION PROCESS

The algae were shade dried and coarsely powdered. About 500 gm powdered drug was extracted successively by cold maceration method with different solvents of increasing polarity i.e. hexane, chloroform, ethyl acetate, ethanol, methanol and water. After 72 hrs of maceration it was filtered. The marc was dried each time before extraction with next solvent. The extracts were concentrated by distilling off the solvent and then evaporated to dryness on water-bath. Color of the extracts was observed and percentage yield was calculated on the air-dried basis **(Table no.4)**

QUALITATIVE PRELIMINARY PHYTOCHEMICAL SCREENING

The extracts were subjected to qualitative preliminary phytochemical screening for identification of the phytochemical constituents and the results was observed in **(Table no.5)**

ESTIMATION OF PHYTO CONSTITUENTS

TANNIN CONTENT (Ansari, 2005)

2gm of sample was extracted with 100ml of distilled water for about 24 hours at room temperature. After 24 hours the mixture was filtered, followed by the addition of 5 ml of saturated lead acetate solution precipitates the tannins as lead tannate. The precipitate was washed with water and dried. Lead tannate was obtained suspended in ethanol. Warmed and decomposed by bubbling in hydrogen sulphite gas. Black precipitate of lead sulphite was removed by filtration and the filtrate concentrated under reduced pressure. The residue obtained was removed by filtration and the filtrate was added with 25 ml of 1% cupuric acetate solution. The precipitate thus obtained was washed, dried and incinerated in a muffle furnace keeping all the material in silica crucible and weighed as cupric oxide. The tannin content was calculated by the following formula, the results are mentioned in table .6

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$$\text{Tannin content} = \frac{(B-A) \times 305}{\text{Weight of drug}} \times 100$$

Where,
B = weight of crucible + material
A= Weight of silica crucible

TOTAL PHENOLIC CONTENT (Schulthess, 1991)

One gram of the powder was extracted in an ultrasonic wave bath with 80mL of aqueous for 2hr. After cooling down, the volume of the solution was adjusted to 100mL. The final solution was centrifuged prior to the colorimetric determination. Tannic acid standards (10, 30 and 50mg) were dissolved in 100ml of aqueous solution respectively. 10ml of Folin-Denis reagent was added to 1 ml of the extract solution or 1mL of standard solution. After reacting for 3 min, 10mL of 35% sodium carbonate solution was added and the test solution was diluted to 100ml with H_2O and mixed. After 45min, an aliquot was centrifuged for 5 min. Then the clear solution was transferred into a cuvette and the absorption coefficient measured at 745nm. The results are mentioned in **table .6**

PHARMACOLOGICAL SCREENING

ACUTE TOXICITY STUDY [8]

The acute toxicity study was carried out by using Wister albino mice of either sex, weighing about 25–30g. This study was performed as per OECD-423 guidelines. Animals were kept in a temp controlled environment (23 ± 2ºC) at 12 hours light/dark cycle and all the animal experiment protocols of this project were approved by the Institutional Animal Ethics Committee. (IAEC, Reg .No.290 / CPCSEA / dated 6-10-08) of Vel's college of pharmacy. In the study, the drug effect was evaluated in a single dose level.
The animals were divided into 4 groups (n=6):
Group I (Control): received 2% CMC (vehicle).
Group II : received Methanolic extract of *Hypnea musciformis* (MEHM) suspended in 2%CMC at a dose of 2000 mg/kg body weight orally.
Group III : received aqueous extract of *Hypnea musciformis* (AEHM) suspended in 2% CMC at a dose of 2000 mg/kg body weight orally

Animals were fasted over night with water *ad libitum*. Food was with held for 3-4 hours after oral administration of the drug. The principles of laboratory animal care were followed. The animals were observed continuously for one hour and observed during 24 hours after administration of the test drug for any changes in general behavior like alertness, aggressiveness, grooming, gripping, touch response, tremors, respiration or other physiological activities like convulsion, lacrimation and writhing etc. [**Table no.7].** At the end of the study the toxicological effect was assessed on the basis of mortality noted after 24 hours

Table 7 Show that the Methanolic extract and Aqueous of *Hypnea musciformis* does not show any marked sign of toxicity and mortality at the dose level 2000 mg/kg body weight orally in mice for 24 hrs and was considered as safe for pharmacological activity. The LD_{50} of the tested extracts might be more.

ANTIOXIDANT

Assay of nitric oxide-scavenging activity: (Nabavi *et al*., 2008). : Sodium nitroprusside (5 mM) in phosphate-buffered saline solution (0.025 M) was incubated with different concentrations (100-1000 µg/ml) of aqueous and Methanolic extract of *Hypnea musciformis* and with standard ascorbic acid. The tubes were incubated at 25°C for 5 hr and diluted with 0.5 ml of Griess reagent and the absorbance of the chromospheres was read at 546 nm. The results are tabulated in **Table .8**

DPPH radical scavenging activity: (Ebrahimzadeh *et al*., 2008). : About 0.1 mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of standard ascorbic acid and to test solutions at the concentrations (100-1000 µg/ml). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm. The results are tabulated in **Table .9**

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ANTI-RHEUMATOID ACTIVITY

The test extracts methanol and aqueous extracts of *Hypnea musciformis* at particular dose of 200mg/kg was selected based on acute toxicity studies and administered orally to animals (rats) to study the anti-rheumatoid arthritic activity of the extracts.

Animals

Wister albino male rats (150-200g) were procured from Tamil Nadu Veterinary College, Chennai, India. They were housed in standard microloan boxes with standard laboratory diet and water *ad libitum.* The experimental protocol was approved by the Institutional animal ethics committee (IAEC), Vel's College of Pharmacy, Chennai. India.

Procedure

Rats were divided into 6 groups

Group I (Control) : Animal were provide with food and distilled water *ad libitum*

Group II (Arthritic control): Complete Freund's adjuvant 0.1ml./rat in right feet paw.

Group III (Standard) : Received Diclofenac sodium (13.5mg/kg.) suspended in vehicle Solution given orally and same time inject in sub-plantar region with Complete Freund's adjuvant 0.1ml./rat in right feet paw of the rats.

Group VI (Test I) : Methanolic extracts (MEHM) (200mg/kg.)(p.o.)

Group V (Test 2) : Aqueous extract (AQHM) (200mg/kg.)(p.o.)

On day 0 Arthritis was induced in animal belonging to group II, III, IV and V by injecting 0.1 ml. of complete Freund's adjuvant (Sigma Chemical Co, USA) below the sub-plantar region of the right feet paw of rats. The next day onwards the Methanolic extract and aqueous extract were administered orally to animal at the dose of 200mg/kg up to 21 days and paw volume was measured at day 4, 8, 14 and 21st. At the end of the experiment period the animals were fasted overnight and sacrificed by cervical decapitation. The blood was collected and serum separated out, for the biochemical evaluation. The percentage inhibition was evaluated using the following formula and results are show on **[Table no .10]**

$$\% I= [1-(\Delta V \text{ treated} - \Delta \text{ control}) \times 100]$$

Where,

I= Percentage inhabitation paw edema

ΔV treated = Mean change in paw volume of treated rat,

ΔV control = Mean change in paw volume of control rat.

BIOCHEMICAL INVESTIGATION [3]

Serum was analyzed for the following biochemical parameter, serum glutamate pyruvate transaminase (SGPT), alkaline phosphates (ALP), Total protein and WBC count **[Table no.11]**

RESULTS AND FINDINGS

- Results of Phytochemical test revealed that aqueous extract of *Hypnea musciformis* showed positive test for Carbohydrate, Glycoside , protein, Amino acids and tannin . Whereas the Methanolic extract of *Hypnea musciformis* showed Carbohydrate, Glycoside, protein, Flavonoid, Phenolic compounds, tannin and Steroid .The selected extract was subjected to free radical scavenging assay by DPPH and Nitric oxide. The Methanolic and Aqueous extract showed significant antioxidant activity in a concentration dependent manner and IC_{50} value was found to be 694µg/ml and 765 µg/ml by DPPH and 590µg/ml and 535µg/ml Nitric oxide method respectively.
- The safety profile of the Methanolic and Aqueous extract of *Hypnea musciformis* was studied by acute toxicity study, the result of study indicating that the mixture is non-toxic and did not produce any untoward effect at the maximum tested dose level.
- The result of Antirheumatoid activity of the Methanolic and Aqueous extract of *Hypnea musciformis* showed significantly decreased arthritic effect at tested dose level of 500 mg/kg and significantly lowers the biochemical parameter SGOT, ALP, Total protein and WBC as compared to Arthritic rat.
- In Histopathological studies the Methanolic extract of *Hypnea musciformis* shows the neither active pannus nor cartilage or bone damage. Whereas the aqueous extract of *Hypnea musciformis* shows cartilage or bone damage as compared the Arthritic control.

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Table .1
Analytical Parameters of *HYPNEA MUSCIFORMIS*

S.No	Parameters	Values (%w/w)
1.	Ash Values	
	Total ash	Not more than 15.02
	Acid insoluble ash	Not more than 1.01
	Water soluble ash	Not more than 8.05
	Sulphated ash	Not more than 3.16
2.	Extractive Values	
	Ethanol soluble extractive value	Not less than O.76
	Water soluble extractive value	Not less than 1.02
	Chloroform soluble extractive	Not less than 0.65
3.	Loss on drying	Not more than 25.96

Table 2
Elemental study of *Hypnea musciformis*

S. No.	Element	Symbol	Result
1.	Calcium	Ca	+
2.	Carbonate	CO_3	+
3.	Chloride	Cl	+
5.	Magnesium	Mg	+
6.	Potassium	K	+
9.	Sodium	Na	+
7	Sulphate	SO_4	-

(+) indicates for presence, (-) indicates for absence

Table .3
Metal analysis of *Hypnea musciformis* powder

S. No.	Metal	Concentration/100g sample
1.	CALCIUM	30.96mg
2.	MAGNESIUM	12.0mg
3.	SODIUM	13.3mg
4.	POTASSIUM	9.21mg
5.	MANGANESE	1.123 mcg
6.	ZINC	0.100 mg
7.	CHROMIUM	In Traces
8.	IRON	5.45 mg
9.	PHOSPHORUS	0.108 mg
10.	SELENIUM	In Traces
11.	IODINE	NIL
12.	COPPER	0.25 mcg
13.	SULPHUR	NIL
14.	CHLORIDE	44.5 mg
15.	MERCURY	NIL
16.	TIN	NIL

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17.	ARSENIC	NIL
18.	LEAD	0.126 mg
19.	NICKEL	NIL
20.	VANADIUM	NIL
21.	CADMIUM	NIL

Table .4

Percentage yields of successive extracts of *Hypnea musciformis*

Extract	Color	Percentage Yield
Hexane extract	Brown	0.134
Chloroform extract	Greenish yellow	0.245
Ethyl acetate extract	Yellowish green	0.120
Ethanolic extract	Brownish black	0.234
Methanolic extract	Greenish yellow	1.80
Aqueous extract	Brown	1.90

Table .5

Preliminary Phytochemical screening of various extracts of *Hypnea musciformis*

Chemical Test	Extracts					
	Hexane extract	Chloroform extract	Ethyl Acetate extract	Ethanolic extract	Methanolic extract	Aqueous extract
Alkaloids						
Mayer's test	-	-	-	-	-	-
Dragendorff's test	-	-	-	-	-	-
Hager's Test	-	-	-	-	-	-
Wagner's Test	-	-	-	-	-	-
Carbohydrates						
Molish's test	-	-	-	-	+	+
Glycosides						
Anthrone test	-	-	-	-	+	+
Borntrager's test	-	-	-	-	+	+
Modified Borntrager's test	-	-	-	-	+	+
Legal's test	-	-	-	-	+	+
Proteins						
Biuret Test	-	-	-	-	+	+
Million's Test	-	-	-	-	+	+
Xanthoproteic test	-	-	-	-	+	+
Amino acids						
Ninhydrin Test	-	-	-		-	+
Saponins						
Foam test	-	-	-	-	-	-

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Flavonoid						
Shinoda test	-	-	-	-	+	-
Phenolic compounds						
Ferric Chloride Solution Test	-	-	-	-	+	-
Lead Acetate Solution Test	-	-	-	-	+	-
Tannins						
Ferric chloride test	-	-	-	-	+	+
Lead acetate test	-	-	-	-	+	+
Gelatin solution test	-	-	-	-	-	+
Terpenoids						
Noller's (or) Salkowski Test	-	-	-	-	-	-
Oil and fats						
Spot test	+	-	-	-	-	-
Steroids						
Liebermann's Burchard test	+	+	-	+	+	-
Salkowski Test	+	+	-	+	+	-

(+) indicates present, (-) indicates absent (Ansari, 2005)

Table .6
Percentage yield of Hypnea musciformis

S.No	Parameters	Results
1.	Total tannins	2.98 % w/w
2.	Total phenolic content	8.75 % w/w

Table .7
Acute Toxicity Study for *Hypnea musciformis* extracts

	Methanolic Extract (2000mg/kg body weight)	Aqueous Extract (2000mg/kg body weight
Aggressiveness	Absent	Absent
Alertness	Present	Present
Convulsion	Absent	Absent
Corneal reflex	Present	Present
Gripping strength	Present	Present

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Lacrimation	Absent	Absent
Pain response	Present	Present
Pinna reflex	Present	Present
Pupils	Normal	Normal
Respiration	Normal	Normal
Restlessness	Present	Present
Righting reflex	Absent	Absent
Salivation	Absent	Absent
Skin color	Normal	Normal
Touch response	Present	Present
Tremors	Absent	Absent
Urination	Normal	Normal
Writhing	Absent	Absent
Mortality	Absent	Absent

Table. 8

Nitric oxide radical scavenging activity *Hypnea musciformis*

Concentration	**% Inhibition**		
	Ascorbic acid	**MEHM**	**AEHM**
100µg/ml	27.49±0.241	23.89±0.68	14.34±0.267
200 µg/ml	36.686±0.419	31.45±0.28	15.87±0.558
400 µg/ml	44.826±0.311	43.01±0.69	23.56±0.152
600 µg/ml	52.92±0.136	50.34±0.81	34.67±0.653
800 µg/ml	67.897±0.425	65.25±0.85	45.67±0.351
1000 µg/ml	81.143±0.065	76.59±0.45	56.89±0.240
IC_{50} Value	565(µg/ml)	694(µg/ml)	590(µg/ml)

The results were expressed as mean of three values.

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Table .9
DPPH radical scavenging activity of *Hypnea musciformis*

Concentration	% Inhibition		
	Ascorbic acid	MEHM	AEHM
100µg/ml	20.297±0.014	18.34±0.51	11.03±0.407
200 µg/ml	32.657±0.313	29.59±0.21	13.34±0.326
400 µg/ml	47.403±0.253	42.87±0.24	25.34±0.970
600 µg/ml	57.052±0.668	52.74±0.59	37.24±0.623
800 µg/ml	72.0840±0.790	68.19±0.87	44.43±0.226
1000 µg/ml	78.261±0.526	69.54±0.23	56.79±020
IC_{50} Value	585(µg/ml)	765(µg/ml)	535(µg/ml)

The results were expressed as mean of three values.

Table-10
Anti- rheumatoid activity in *Hypnea musciformis* extract

Group	Mean Change in paw edema (Mean ± SED				Average % inhibition of paw edema
	Day 4th	Day 8th	Day 14th	Day 21st	
Control	3.8±0.09	4.02±0.06	4.12±0.12	4.62±0.11	-
Arthritic control	3.7±0.02	3.86±0.47	3.90±0.05	4.34±0.32	19%
Standard	4.19±0.07	4.27±0.03	4.7±0.23	4.5±0.02	78%
MEHM	**4.0±0.05	** 4.29±0.02	* 4.1±0.01	**4.34±0.2	62%
AEHM	*4.35±0.12	*4.38±0.16	*4.5±0.02	4.34±032	40%

CFA- Complete Freunds' adjuvant
MEHM- Methanolic extract of *Hypnea musciformis*
AEHM- Aqueous extract of *Hypnea musciformis*
N =6 Symbol represent statistical signification, Arthritic control Vs Test a=p<0.01, b=p<0.01, Standard Vs Test **p<0.01, * P<0.05.Data analyzed by one way ANOVA followed by Dunnetts test.* indicate the level of significant, a-used to indicate significant between Arthritic control Vs Test -used to indicate the significance between Standard Vs Test

Table .11
Effect of Hypnea musciformis extract on biochemical parameters against CFA induced arthritis in rat

Treatment	Dose (Mg/kg.)	SGPT ($UL^{-}1$)	ALP ($UL^{-}1$)	Total Protein (mg. %)	WBC. Count/ml.
Control I-.	1%CMC	280.53±7.74	392.20±10.90	8.37±0.23	10409±249.7

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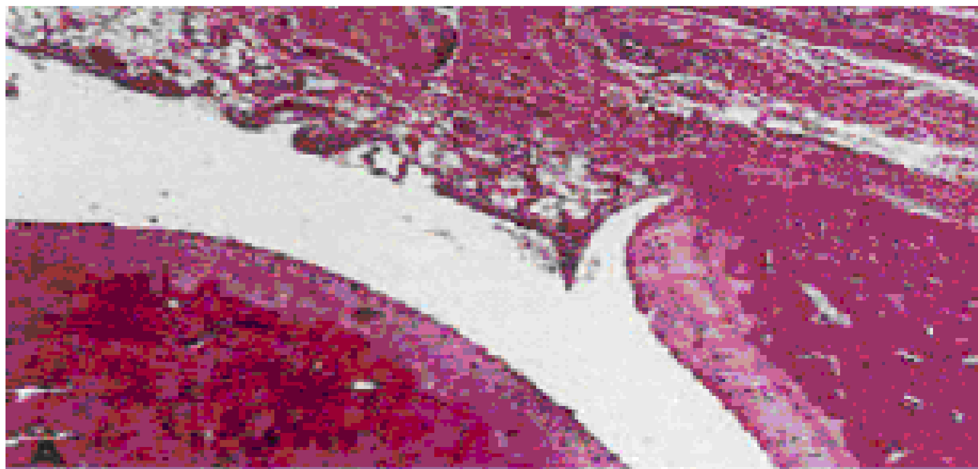
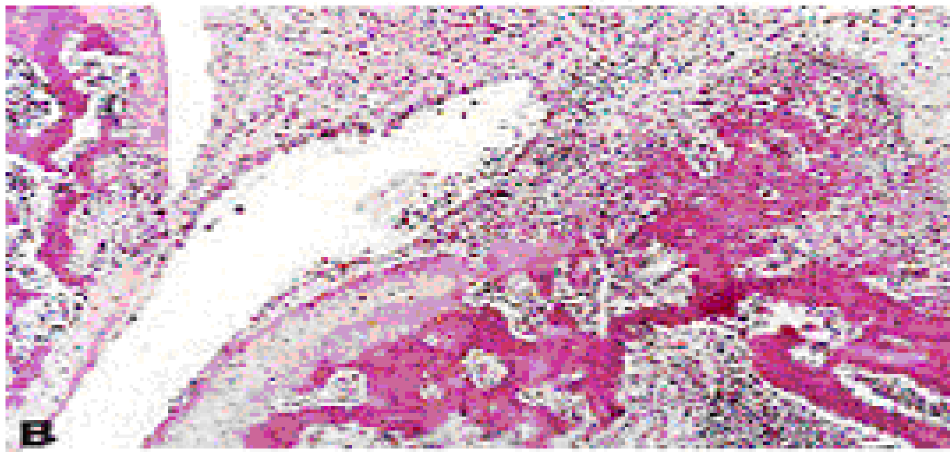
Arthritic Control II	0.1ml./rat	265.24±3.9 4	309.76±1.97	7.49±8.23	7891±9.77
Group III- Standard	13.5mg/kg.	192.8575	275.54±4.19	6.70±0.09	5951±150.6
Test-1 (MEHM)	200mg./kg	a** 224.0±36	a** 313.85±5.61	a** 7.22±0.11	a** 8977±107.8
Test-2 (AEHM)	200mg/kg.	b** 134.78±4.	b** 348.23±5.35	b** 5.09±0.120	b** 6543±71.65

CFA- Complete Freunds' adjuvant
SGPT- Serum glutamate pyruvate transaminase
ALP Alkaline phosphates
T.P.-Total protein
WBC count,
MEHM- Methanolic extract of *Hypnea musciformis*
AEHM- Aqueous extract of *Hypnea musciformis*
N=6, Arthritic Control Vs test a=$p<0.01$, b=$p<0.05$.Standerd Vs Test **$p<0.01$, **$p<0.05$ Data analyzed by one way ANOVA followed by Dennetts test

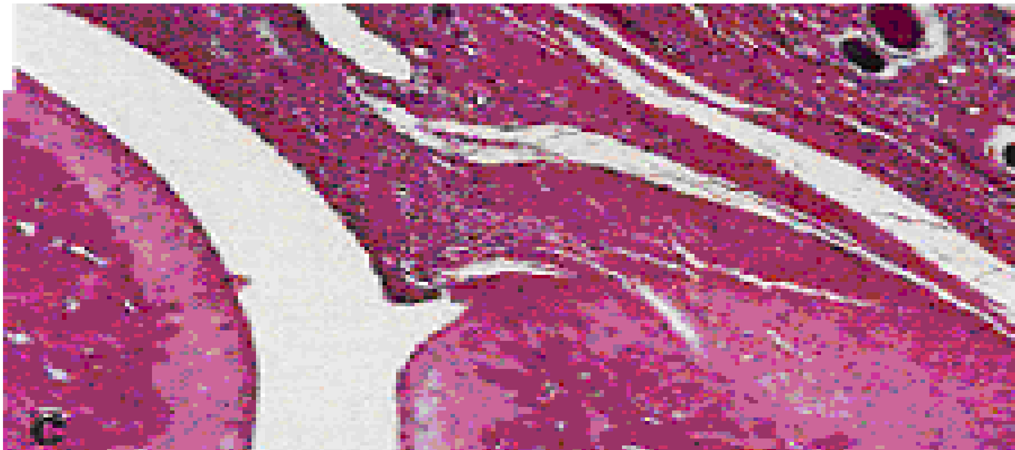
Histopathological study



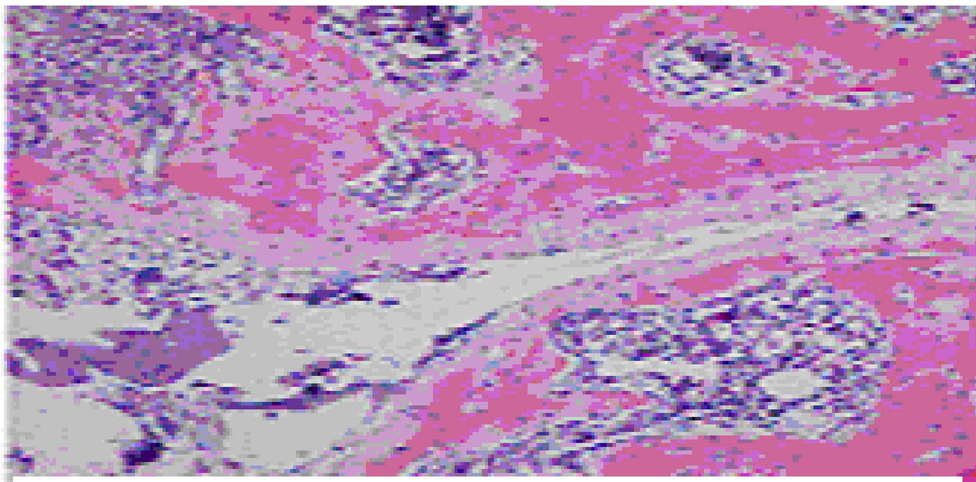
(A) Normal rat joint (no Freund's complete adjuvant [FCA]);



(B)Joint damage in the rat adjuvant arthritis model



(C) Joint from MEHM/FCA-treated rat on day 35. Neither active pannus nor cartilage or bone damage is seen



(D) Joint from AEHM/FCA-treated rat on day 35. cartilage or bone damage is seen

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Histopathological findings:
Hind paw tibiotarsal joints at site of attachment to the anterior aspect of the tibia (hematoxylin and eosin stained, original magnification × 40);

- Normal rat joint (no Freunds complete adjuvant [FCA]);
- Joint from vehicle/FCA-treated rat on day 35. Synovitis with pannu formation is seen exostosis seen at joint margin;
- Joint from MEHM/FCA-treated rat on day 35. Neither active pannus nor cartilage or bone damage is seen
- Joint from AEHM/FCA-treated rat on day 35 cartilage or bone damage is seen

MEHM – Methanolic extract of *Hypnea musciformis*

AEHM- Aqueous extract of *Hypnea musciformis*

DISCUSSION AND CONCLUSION

- **In Physico chemical analysis** the ash value was found to be 15.02 of total ash, 1.01of acid insoluble ash and 8.05 of water soluble ash and the sulfated ash 3.10. Extractive values are, expressed in **[Table no.1]** as 0.76 alcohol, 0.65chloroform, and 1.02 water. Loss on drying is 25.96

- **In Inorganic analysis** the study of inorganic element on *Hypnea musciformis* was showed the presence of chloride, calcium, sodium and carbonate, magnesium, and potassium are absent, and results are expressed in **[Table no.2]**

- **In Metal analysis** of *Hypnea musciformis* showed the presence that calcium, magnesium, sodium, potassium, manganese, zinc, iron, phosphorus, copper, chloride and lead are present, other compound like chromium and selenium is present in trace amount .Iodine, sulphur, mercury, tin, arsenic, nickel, vanadium, cadmium are absent. The yield was shown in the **[Table no.3]**

- **In Extraction** the successive extraction was done in the order of increasing polarity i.e. hexane, chloroform, ethyl acetate, ethanol and water. The yield was reported in **[Table no.4].**

- **In preliminary Phytochemical analysis** was carried out for the identification of various constituent. It answers positively for carbohydrate, glycosides, tannins, protein, Phenolic compound and steroid which is reported in **[Table no.5].**

- **The Percentage yield of Total tannin content and Total phenolic content** in Hypnea musciformis in was reported in **[Table no.6].**

- **In Acute Toxicity**, **Table. 7** Show that the Methanolic extract and Aqueous of *Hypnea musciformis* does not show any marked sign of toxicity and mortality at the dose level 2000 mg/kg body weight orally in mice for 24 hrs and was considered as safe for pharmacological activity

- **The Antioxidant activity** was carried out using the *In – vitro* model .Methanolic and Aqueous extract of *Hypnea musciformis* contains poly phenols (tannin), a electron donor and could react with free radical to convert them to more stable product and terminate radical chain reaction **(Oakley, 1998).** Maximum percentage of free radical scavenging property proved by DPPH and Nitric oxide method. IC_{50} value was found to be 694µg/ml and 765 µg/ml by DPPH and 590µg/ml and 535µg/ml Nitric oxide method respectively which was reported in **[Table no.8 , 9].**

- **The Antirheumatoid activity** was carried out using Freund's complete adjuvant [FCA] model. The tested extracts significantly lower the synovial damage during the experimental period, which were significant when compared to the arthritic vehicle treated group. The maximum effect was observed at the end of the21th day which was reported in **[Table no.10].**

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- **On biochemical parameters**, Methanolic and Aqueous extract significantly lowers SGOT, ALP, Total protein and WBC as compared to Arthritic rat. [**Table no.11].**

- **In Histopathological studies** Methanolic extract of Hypnea musciformis shows effective in curing damage as compared to aqueous extract and arthritic control

SIGNIFICANCE OF THE WORK:

Methanolic and Aqueous extract of *Hypnea musciformis* showed significant antioxidant activity in a concentration dependent manner. The result of Antirheumatoid activity of the Methanolic and Aqueous extract showed that arthritic level was significantly decreased in arthritic rats at tested dose levels (500 mg/kg) and significantly lowers the biochemical parameter SGOT, ALP, Total protein and WBC as compared to Arthritic rat.

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