Research Article

WOUND HEALING ACTIVITY OF SPIRULINA EXTRACTS

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ABSTRACT

Spirulina is a cyanobacterium, a, blue green algae belong to cyanophyceal, a, procaryatic form. The wound healing activity of different extracts of spirulina was carried out using excision wound models. Petroleum ether, chloroform and methanol extracts showed significant wound healing. Here the standard drug is 0.2% w/w nfz. Ointment.

Keywords: Inflammatory process, wound strength, substrate phase, proliferative phase, remodeling phase. Wound contraction.

INTRODUCTION

Spirulina is a cyanobacterium, a, blue green algae belong to cyanophyceal, a, procaryatic form. The photosynthetic pigment phycocyanin and phycoerythrin makes it to be included in cyanophycean algal group, but the nature of it's nucleus (procaryatic) engrouped it in as a prokaryotic organism a cyanobacterium. In other wards spirulina is a photosynthetic prokaryotic microorganism. It is a simple, microscopic blue green algal. It grows naturally in fresh, brackish, sewage water and even in saline environment. It grows through photosynthesis, hence, can be termed as vegetative food. It has been already affectively promoted as a natural food.

MATERIALS AND METHODS

The shade dried spirulina was powdered & was extracted in a soxhlet's apparatus. The powdered drugs (500 gm) were charged into soxhlet apparatus each time for each solvent. The soxhlet apparatus was kept on a heating mantle, to provide constant temperature to the process. The extraction was carried out successfully with petroleum ether, chloroform & methanol. After completion of extraction the concerned solvents were removed under reduced pressure and the % of yield was 41, 26 & 55 respectively for pet ether extract, chloroform extract and methanol extract respectively. Each of the extract was subjected to chemical tests and pharmacological screening[1-4] for wound healing activity.

Acute Toxicity study/ Max. Tolerated Dose

Acute, Toxicity study was carried out as per the stair case method[5] as per the OECD guideline 425. With reference IAEC CPS, Mohuda bearing the Regd. No. 1170/ac/2008/CPCSEA

Wound Healing

Wound is a loss or breaking of cellular and anatomic or functional continuity of living tissue[6]. Injury of the skin induces repair mechanism that restores its functions in protecting individual against environmental factors that might be harmful. Wound healing is the process of repair[7] that follows injury to the skin and other soft tissues. It is a complex phenomenon involving a number of processes including induction of an acute inflammatory process, regeneration of parenchymal inflammatory process[8], migration and proliferation of both parenchymal and connective tissue cells, synthesis of extracellular matrix (ECM) proteins, remodeling of connective tissue and parenchymal components and acquisition of wound strength[9]. All these steps are orchestrated in a controlled manner by a variety of cytokines including growth factor[10]. The different phases constitute the physiologic process of wound healing

i) Substrate phase[11]
ii) Proliferative phase[11]
iii) Remodeling phase[11]

Some of these growth factors like platelet derived growth factor (PDGF), transforming growth factor B (TFG-B), fibroblast growth factor (FGF) and epidermal growth factor (EGF) etc. have been identified in healing of wounds. In chronic wounds normal healing process is disrupted and in such cases some growth promoting agents exogenously applied or some compounds which can enhance the in situ generation of these growth factors required to augment the healing process[12]. Several factors delay or reduce wound healing including bacterial infection necrotic tissue, interference with blood supply, lymphatic, blockage and diabetes mellitus. Generally if the said factor could be inhibited or controlled by any agent, increasing healing rate could be achieved[13].

Animals and the wound healing activity

With reference to IAEC (Institutional Animal Ethical Committee) CPS, Mohuda, bearing the registration No 1170/ac/08/CPCSEA. Healthy Wistar rats (150-180 gm) of either sex were selected and they were obtained from animal house CPS, Mohuda. The rats were maintained at well ventilated, temperature controlled (30^0C) in animal

room for 7 days prior to the experiment. The animals were provided with normal food and water adlibitum. The rats were periodically weighed before and after the experiment. The rats were anaesthetized prior to the infliction of experimental wound by light ether. The surgical intervention was strictly carried out under sterile condition. Rat showing any sign of infection were excluded from the study. Wounding was performed aseptically in Excision[2] wound models in wistar rats for assessing the wound healing activity. All the animal experimental protocol has been approved by the IAEC bearing Regd. No 1170/ac/08/CPCSEA.

A full thickness of the excision wound of 2.5cm in the width (circular area 4.90 cm^2) and 0.2 cm depth was created along the markings by picric acid. The entire wound left open[14]. The animals were divided into eleven groups six in each (n = 6)

The group I animals were treated with simple ointment base (control)

Group II were treated with a reference standard 0.2% w/w Nitrofurazone (NFZ)

Group III, IV, V, VI, VII, VIII, IX, X, XI were treated with 3%, 7% and 10% w/w petroleum ether, chloroform and methanol extract ointments respectively for 16 days. The extract ointments (3, 7, 10% w/w) at a quantity of 0.3 gm were applied once daily to treat different groups of animals. The simple ointment base and 0.2% w/w NFZ ointment were applied in same quantity to serve as a control and standard respectively. Wound healing potential was monitored by wound contraction and wound closure time. The wound contraction was calculated as the percentage reduction in wound area. The progressive change in wound area were monitored planimetrically using transparent paper and permanent marker on 4^{th}, 8^{th}, 12^{th} & 16^{th} day following the initial wound. The recorded wound area was measured with graph paper.

RESULTS

Phytochemical investigations of different extracts showed the presence of alkaloids, tannins and phenolic compounds, steroids in PEFS, where as alkaloids, cardiac glycosides, Flavones and Flavonoids are present in PEFS, CFS, MFS. Saponin is present in MFS and steroids & sterols present in CFS. The dose of the extracts were found to be 300 mg/kg b.w. derived from maximum tolerated dose with reference to IAEC, bearing the Regd. No 1170/ac/08/CPCSEA. The level of significance was $P<0.05$, $P<0.01$, $P<0.001$. In this excision model study, significantly improved wound-healing activity has been observed with PEFS, CFS and MFS compared to that of the reference, standard (NFZ) and control group of animals and the healing capacity was in the order of PEFS>CFS>MFS. A photographic representation of the entire wound healing process is done beginning form the 0 day treatment up to 16 day treatment with this research paper (table 1, table 2 and figure 1) .

DISCUSSION

The effect of the extract ointments, NFZ ointment (standard) and simple ointment base (control) in the excision wound model was assayed by measuring the wound area and wound contraction respectively. The present investigation revealed that the test extract in varying concentrations in the ointment base were capable of producing significant wound healing activity. The preliminary phytochemical analysis of the tested extracts of spirulina revealed the presence of flavonoids, alkaloid & triterpinaids. Triterpinoids[15] and flavonoids[16] are known to promote the wound healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelisation.

Flavonoids are known to reduce lipid peroxidation not only by preventing or inhibiting lipid peroxidation it is believed to increase the viability of collagen fibrils by promoting DNA synthesis[17]. Thus wound healing property of spirulina may be attributed the phytoconstituents present in it, which may be due to their individual or additive effect that fastens the process of wound healing. Between these three extracts, only petroleum ether extract showed better wound healing activity in comparison to that of CFS and MFZ. It may be due to the presence of triterpinoids, flavonoids, steroids, Tannins and phenolic compounds in the petroleum ether extract.

Table 1: Wound area in different post wound days of rats treated with different extracts of spirulina (n = 6)

Excision wound model, wound area mm^2 ±SEM post wound days					
Treatment	**0**	**4**	**8**	**12**	**16**
Simple ointment base	498.81±2.51**	385.64±1.36**	276.16±1.61**	176.32±1.51**	74.12±1.61**
Standard (0.2% w/w) NFZ ointment	496.50±2.81**	256.16±1.56**	169.32±1.91**	52.12±1.64**	0
Pet. Ether extract ointment (3% w/w)	495.30±2.01*	402.61±1.81**	302.11±1.41**	91.28±1.03**	28.10±1.14**
Pet. Ether extract ointment (7% w/w)	494.32±0.86*	309.12±1.61**	285.16±1.04**	82.01±0.98*	22.00±0.95*
Pet. Ether extract ointment (10% w/w)	491.16±1.16**	382.16±1.06**	208.00±0.98*	54.64±0.76*	6.18±0.96*
Chloroform extract ointment (3% w/w)	498.81±2.17**	446.57±2.16**	305.26±1.41**	94.12±1.31**	34.81±1.61**
Chloroform extract ointment (7% w/w)	495.26±1.63**	433.51±1.06**	281.83±0.91*	84.40±1.21**	26.51±1.21**
Chloroform extract ointment (10% w/w)	498.12±1.16**	408.53±0.75*	278.26±1.11**	66.63±0.93*	9.01±0.49*
Methanol extract ointment (3% w/w)	499.49±2.03**	437.46±0.72*	306.02±1.12**	97.03±1.21**	41.06±1.21**
Methanol extract ointment (7% w/w)	489.90±0.81*	440.26±0.96*	375.01±1.12**	87.60±0.71*	31.61±0.81*
Methanol extract ointment (10% w/w)	491.81±0.46*	402.60±0.61*	316.61±0.37*	74.03±0.61*	9.36±0.61*

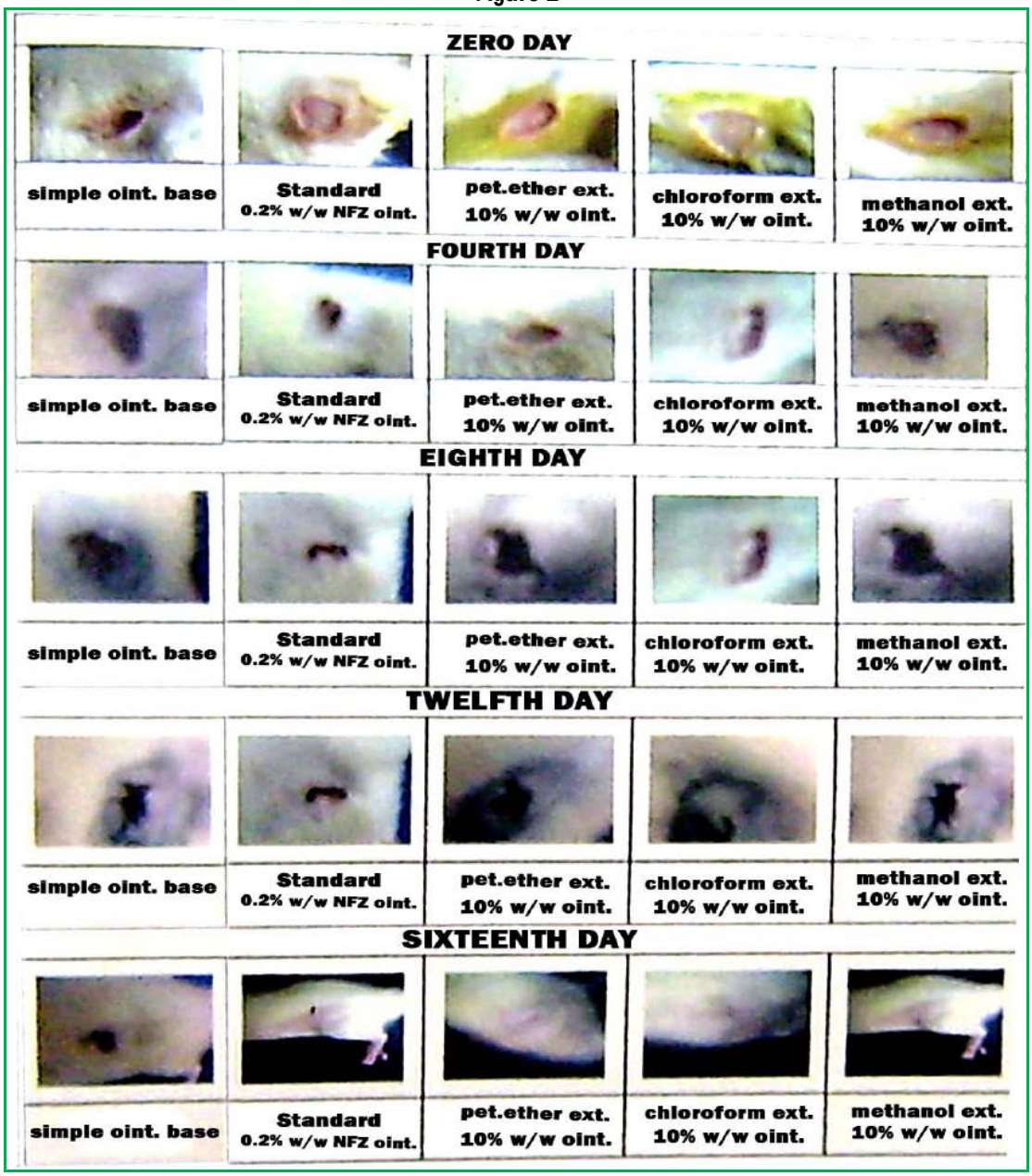
Results expressed in Mean ± SEM for (n = 6) **P< 0.05 *P < 0.001 **P < 0.01

Table 2: Excision wound model percentage of wound contraction activity of spirulina

Excision wound model Percentage of wound contraction post wounding days					
Treatment	**0**	**4**	**8**	**12**	**16**
Simple ointment base	0	22.68±0.196**	44.64±0.235*	64.72±0.276*	85.14±0.168***
Standard (0.2% w/w) NFZ ointment	0	48.41±0.282*	65.90±0.284*	83.50±0.163***	100
Pet. Ether extract ointment (3% w/w)	0	18.71±0.224*	39.00±0.256*	81.57±0.214*	94.33±0.193**
Pet. Ether extract ointment (7% w/w)	0	31.16±0.276*	42.31±0.218*	83.41±0.198**	95.55±0.197**
Pet. Ether extract ointment (10% w/w)	0	22.19±0.293*	51.65±0.196**	88.82±0.156***	98.74±0.162***
Chloroform extract ointment (3% w/w)	0	10.47±0.218*	38.80±0.195**	81.71±0.235*	93.02±0.196**
Chloroform extract ointment (7% w/w)	0	12.47±0.152***	43.09±0.286*	82.96±0.254*	94.65±0.199**
Chloroform extract ointment (10% w/w)	0	17.99±0.193**	44.14±0.196**	86.62±0.192**	98.19±0.163***
Methanol extract ointment (3% w/w)	0	12.42±0.172***	38.73±0.201*	80.57±0.258*	91.78±0.187**
Methanol extract ointment (7% w/w)	0	10.13±0.162***	23.45±0.322*	80.11±0.156***	93.55±0.203*
Methanol extract ointment (10% w/w)	0	18.14±0.231*	35.62±0.312*	84.95±0.104***	98.09±0.165***

Results expressed as Mean ±SEM for six observations *P<0.05, **P<0.01, ***P<0.001.
Percentage of wound contraction was calculated by using the formula.
Initial wound area - wound area in post wounding days/ initial wound area.

Figure 1



CONCLUSION

In this excision model it was studied that there is a significant improvement in the wound healing activity has been observed with PEFS, CFS and MFS compared to that of the reference standard and control group of animals and the healing capacity was in order of PEFS>CFS>MFS. The PEFS showed 98.74% and standard drug showed 100% wound contraction on 16th day.

The effect of extract ointments, NFZ ointment (standard) and simple ointment base (control) in excision wound model was assayed by measuring the wound area and wound contraction respectively. The present investigation revealed the test extracts in varying concentrations in the ointment base were capable of producing significant wound healing activity.

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