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Research Article

ANTI LEECH ACTIVITY OF CHITOSAN AND CHITOSAN CHLORHYDRATE AGAINST PLACOBDELLA EMYDAE

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ABSTRACT

Background: In different National Pharmacopeias drug for treating complications with leech are not registered.

Methods: Antileech activity of Chitosan and Chitosan chlorhydrate (CCH) on Placobdella emydae was examined and compared with Albendazole and Mebendazole as control and distilled water as placebo in terms of mean paralyzing time and mean death time.

Results: DSC displayed absence of endothermic peak and glass transition temperature; depicting amorphous nature of Chitosan and CCH. Both Chitosans showed dose dependent activity. Albendazole most effectively killed leeches after 32.5±3.62 min at 2% concentration while 2% Mebendazole took 46.5±3.64 min. Chitosan (2, 3 and 4%) and CCH (1%, 1.5% and 2%) showed 3^+ antileech impacts. While Chitosan (5%) and CCH (2.5% and 3%) showed 4^+ impact indicating antileech activity. 1% of Albendazole, Mebendazole and 3% CCH showed statistically similar results ($p<0.001$).

Conclusion: High affinity to in-vivo macromolecules might be cause of antileech property possessed by Chitosan products. Both Chitosan forms were amorphous leading to easily enter in parasite body. Possible reason for better performance of CCH could be better transcuticular absorption into parasite.

Future Research Implications: Chitosan and CCH can be checked for veterinary application as a de wormer in cattle, pigs and sheep.

Social Implications: Being biodegradable, biocompatible, non toxic, physiologically inert and in amorphous form, CCH can be used as de worming product or as an effective supplement for medicinal products.

Originality/value of paper: Being not having any effective drug, without side effect and cost benefits; CCH proves to be a solution.

KEYWORDS: *Antileech activity, Chitosan, Albendazole, Mebendazole, Biodegradable polymers*

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INTRODUCTION

Leeches are not true worms, but they are described as parasitic worms. They are segmented worms that belong to the phylum Annelida and comprise the subclass Hirudinea which are external parasites of humans, domestic and wild animals[1]. Like the oligochaetes, such as earthworms, leeches share a clitellum and are hermaphrodites[2].

Leeches live in hosts and can cause anaemia and may act as vectors of animal pathogens. The main symptoms included pain, haemoptysis, snoring, dyspnea, cough, dysphagia, anaphylaxia and bleeding from vagina[3, 4]. Serious complications are expected like dyspnoea, hemoptysis[5] or hematemesis and bleeding from vagina[6]. The leeches can transmit some bacteria, viruses and parasites. Some lethal

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diseases such as AIDS, Hepatitis B and Toxoplasmosis can be transmitted by leeches[7,8,9].
The leeches mainly inhabit in ponds, lakes and streams. Land leeches include Haemadipsa zylanica Haemadipsa sylvestris, Hamadipsa picta, while Aquatic leeches include Limnatis nilotica, Myxobdella africana, Dinobdella ferox, Phytobdella catenifera and Teromyzon tessulatom[10,11].

Placobdella emydae are Translucent, having elliptic body with head region dilated, coma shaped having grayish-green color with posterior sucker narrower than the widest part of the body. They have three pairs of papillae on dorsal surface, intermediate pair being largest. They tend to breed in May-July[4].

In some cultures hypertonic fluids of acetic acid and Lidocaein have been used for removing leech[12]. Albendazole and Mebendazole are originally used as an anthelmintic to treat worm infestations in both humans and animals. Some of their commercial preparations are intended for veterinary use as a de wormer in cattle, pigs, and sheep. They cause degenerative alterations in the intestinal cells of the worm by binding to the colchicine-sensitive site of tubulin, thus inhibiting its polymerization or assembly into microtubules.

In different National Pharmacopeias in the world drug for treatment of complications with leech are not registered. No effective drug, without any side effect and with appropriate cost benefit, has been offered for leeches.

The present research is thus motivated by the need to find new substances derived from nature which possesses anti leech activity with a low degree of toxicity for humans.
Chitosan is a linear polycationic copolymer of β (1–4) linked 2-acetamido-2-deoxy-b-D-gluco-pyranose and 2-amino-2-deoxy-b-D-glucopyranose obtained from deacetylation of chitin, a structural polysaccharide which is very abundantly distributed in nature. Chitosan have demonstrated antiviral, antibacterial, antifungal and other medicinal properties[13].

In recent years chitosan has gained increasing interest in the pharmaceutical field due to its favorable biological properties such as biocom-patibility, biodegradability, and lack of toxicity, together with its wide availability, low cost and high versatility of use. Hence, it is used as an adjuvant in various drug delivery systems[13].

The current study was designed to examine the antileech activity of Chitosan and its water soluble form Chitosan chlorhydrate on Placobdella emydae.

MATERIALS

2.1 Chitosan

Chitosan and Chitosan Chlorhydrate were obtained as gift sample from Mahatani Chitosan Pvt. Ltd., Ahmedabad, India.

2.2 Leeches

In this study, 80 Placobdella emydae leeches with 10-20 mm length, were selected from spring waters from Satara district (Maharashtra, India). Translu-cent, elliptical and grayish green colored body, dilated head region along with three pairs of papillae on dorsal surface and anterior sucker with shallow anterior cup were the main signs for detection of Placobdella emydae species. They were washed with water to remove dirt.

2.3 Chemicals

Albendazole and Mebendazole were obtained as gift sample from Micro Lab., Goa. All the chemicals and reagents required for the present research work has been obtained from authentic supplier and were of analytical grade. Triple distilled water obtained from triple distillation apparatus at Govt. College of Pharmacy Karad was used throughout the experiment.

METHODS

3.1 Authentication of Chitosan and Chitosan Chlorhydrate by Differential Scanning Calorimetry (DSC)

Differential scanning calorimetric (DSC) analyses of the samples were carried out by using differential scanning calorimeter equipped with computer analyzer (Shimadzu TA –60 differential scanning calorimeter, Shimadzu Corporation, Kyoto, Japan). Samples (of 3-7 mg) were heated under nitrogen atmosphere on an aluminum pan at a heating rate of 10 °C/min over the temperature range of 20°C - 300°C.

3.2 Preparation of the Drugs

The drugs Mebendazole and Albendazole were investigated and compared with distilled water as negative control. Powdered drugs were diluted in 10 ml distilled water and then were added to glass container.

3.3 The anti-leech assay

Chitosan (1, 2, 3, 4 and 5%) and Chitosan chlorhydrate (1, 1.5, 2, 2.5, 3%) were used as the test treatment. First, leeches were put individually in a glass container with 600ml distilled water. Then Chitosan, Chitosan Chlorhydrate and drugs were added and their effects were screened for 720 min, and time to paralyze, kill, and death of each leech was recorded. Albendazole and Mebendazole were used as control and distilled water as the placebo group which investigated Placobdella emydae using anti-leech assay. Time for paralysis was noted when no movement of any sort could be observed except the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50°C.The low average paralyzing and killing time of these compounds reflects anti-leech properties.

The severity of effect of these compounds/drugs based on time was categorized into five groups:

(1) 4+→ paralysis and death of each leech within 1–60 min after addition of the drug,

(2) 3+→ paralysis and death of each leech within 61–120 min after addition of drug,

(3) 2+→ paralysis and death of each leech within 121–180 min after addition of drug,

(4) 1+→ paralysis and death of each leech within 181–240 min after addition of drug,

(5) Negative → paralysis and death of each leech within 241–720 min after addition of drug.

The efficacy of the drugs which were able to kill leeches within 1–60 min after addition reflects the anti-leech properties of these compounds, and therefore, they may be used in the treatment of infestation with Placobdella emydae in the future[14].

3.4 Data Management and Statistical Analysis

Comparison of control and treated groups was performed by one-way ANOVA. All statistical analysis was performed by using statistical software Instat 3.1. The difference between the means was considered significant at $p < 0.001$.

RESULTS AND DISCUSSION

Chitosan has received considerable attention as possible pharmaceutical excipient in recent decades. Chitosan possesses antibacterial, antifungal, antioxidant, free radical scavenging, sun screening, wound healing and moisturizing properties too. Chitosan derivatives are widely employed in surgical sutures, dental implants, artificial skin, bone rebuilding, corneal contact lenses, controlled release drug delivery and material encapsulation[13]. In this study, anti-leeches effect of Chitosan and Chitosan Chlorhydrate were studied in comparison with two strong antiparasitic drugs Albendazole and Mebendazole.

The DSC thermograms of Chitosan and water soluble Chitosan showed absence of any endotherm indicating its amorphous nature (Fig 1). Rather both Chitosan as well as water soluble Chitosan displayed Tg (glass transition temperature) in the range of 120 – 150 ° C. The presence of Tg in the thermogram confirmed amorphous nature of both Chitosan and its derivative[15].

In the present study, anthelmintic (anti-leech) activity of Chitosan and its water soluble derivative Chitosan Chlorhydrate is studied and demonstrated against Placobdella emydae and is depicted in Table 1.

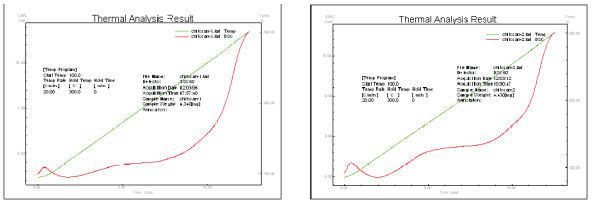

Fig.1: DSC thermograms of Chitosan and Chitosan Chlorhydrate

Table 1: Antileech activity against Placobdella emydae expressed as paralysis time (min.) and death time (min.)

TEST	Concentration (%)	Time taken for paralysis (Min.)	Time taken for Death (Min.)	Influence
Chitosan	1	122.1 ± 2.66	135.4±4.52	2+
Chitosan	2	88.4±2.83	98.6±2.56	3+
Chitosan	3	78.6±1.58	84.8±2.14	3+
Chitosan	4	66.7±1.46	73.3±2.12	3+
Chitosan	5	44.5±2.13	53.0±2.36	4+
Chitosan Chlorhydrate	1	82.4±3.63	103.9±2.98	3+
Chitosan Chlorhydrate	1.5	74.2±2.43	88.6±3.44	3+
Chitosan Chlorhydrate	2.0	57.5±2.75	67.6±2.87	3+
Chitosan Chlorhydrate	2.5	34.5±2.34	46.6±2.43	4+
Chitosan Chlorhydrate	3	31.4±2.46	42.8±1.96	4+
Albendazole	1	29.3±1.79	46.4±2.34	4+
Mebendazole	1	30.4±1.46	49.5±3.1	4+
Albendazole	2	20.4±2.33	32.5±3.62	4+
Mebendazole	2	23.6±2.54	46.5±3.64	4+
Distilled Water	---	720±0	---	-ve

Anti-leech tests are reported based on the mean time to death with nine times repetitions and as mean±standard deviation. In this study, Albendazole had the most effective Anti-leech activity it killed leeches after 32.5±3.62 min at 2% concentration. It was found that maximum concentration of Mebendazole killed leeches after 46.5±3.64 min.

Albendazole and Mebendazole (1% and 2%) killed leeches with the 4+ intensity and had strong effect on mortality of Placobdella emydae.

Impact of distilled water was extremely negative for both paralysis and death time. Paralysis and death properties revealed that both forms of chitosan at different doses have different potential, increasing with dose. Chitosan (2, 3 and 4%) and Chitosan Chlorhydrate (1%, 1.5% and 2%) showed 3^+ anti leech impact. While Chitosan (5%) and Chitosan Chlorhydrate (2.5% and 3%) showed 4^+ impact and hence possess anti leech activity.

Collected data was subjected for statistical analysis for various parameters to find out significant differences.

Test between all groups

1. **Mean paralysis Time**

Data of 1% and 2% concentrations of all groups were compared and significant differences were observed between both Chitosan forms and drugs under test at $p < 0.001$.

2. **Mean Death Time**

Data of 1% concentration of all groups were compared and significant differences were observed between both Chitosan forms and drugs under test at $p < 0.001$.

Test between main groups (Chitosan, Chitosan Chlorhydrate, Albendazole & Mebendazole)

1. **Mean paralysis Time**

Data of 1% and 2% of Albendazole, Mebendazole and 2.5% and 3 % Chitosan Chlorhydrate were compared and significant differences were observed between 2% of Albendazole, Mebendazole and 2.5% Chitosan Chlorhydrate at $p < 0.001$.

But 1% of Albendazole, Mebendazole and 3% Chitosan Chlorhydrate were found to show similar results at $p < 0.001$.

2. **Mean Death Time**

Similar results to that of mean paralysis time were obtained for mean death time and 1% of Albendazole, Mebendazole and 3% Chitosan Chlorhydrate were found to show similar results at $p < 0.001$.

This indicates that paralysis and death effect on leeches by 3% Chitosan Chlorhydrate were relatively similar in comparison to two anti-parasite drugs. Though the highest tested concentration of 5% Chitosan showed anti leech effect but the results are not comparable with positive control groups. These results suggest that other derivatives of Chitosan should be examined on Placobdella emydae and other leeches for possible anti leech effects.

It seems that high affinity to in vivo macromolecules; remarkably to proteins might be the cause of anti leech property possessed by Chitosan products. Both Chitosan forms were in amorphous forms as concluded from DSC analysis. And amorphous forms reported to have good membrane permeability leading Chitosan to enter easily in parasite body[16]. The possible reason for the better performance of Chitosan chlorhydrate compared to Chitosan could be due to easy transcuticular absorption of Chitosan chlorhydrate into the body of the parasite more than the Chitosan.

CONCLUSION

The results showed that Chitosan Chlorhydrate possess anti leech effects above 2.5% concentration and are comparable to Albendazole & Mebendazole 1% concentrations. 5% Chitosan has a weak effect on Placobdella emydae. Chitosan chlorhydrate had demonstrated significantly better performance than the Chitosan at various test stages, might be due to better transcuticular absorption in worms due to higher water solubility. Being biodegradable, biocompatible, non toxic, physiologically inert and in amorphous form, Chitosan Chlorhydrate can be used as de worming product or as an effective supplement for medicinal products.

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