ejpmr, 2019,6(2), 608-613

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EFFECT OF WHOLE CELL AND OUTER MEMBRANE PROTEIN VACCINES ALONG WITH IMMUNOADJUVANT *ANDROGRAPHIS PANICULATA* AGAINST STAPHYLOCOCCOSIS DISEASE IN FINGERLINGS OF *LABEO ROHITA*

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Article Received on 21/12/2018 **Article Revised on 11/01/2019** **Article Accepted on 31/01/2019**

ABSTRACT

Whole cell and Outer membrane protein vaccines, when administered with immunoadjuvant increases the efficacy of the vaccine uptake and boosts the innate immune system of fish. The present study is to develop the whole cell and outer membrane protein vaccine, administered along with immunoadjuvant *Andrographis paniculata* in *Labeo rohita* fingerlings, post challenged with virulent *Staphylococcus aureus*. 25 fingerlings were pre-treated with hyper- osmotic solution of 2% NaCl and vaccinated twice with booster dose on day 1 and second dose on day 30. After 30 and 60 dpv (days post vaccination) fingerlings were bath challenged for up-to 1 hour in the lethal dose of $1x10^8$ cells/ml. SDS-PAGE results revealed, WC vaccine with band length of 27.95 KDa and OMP vaccine with band length of 27.43-35.43 KDa respectively. Total protein concentration of WC vaccine was found to be 34µg/ml and 22µg/ml in OMP vaccine respectively. After 30 and 60 dpv there was significant increase in RPS rate of experimental groups, showing a RPS 84% in OMP and 92% in OMP+A when compared to a RPS of 76% in WC and 80% in WC+A respectively. The results inferred that, both OMP and WC vaccine along with an immunoadjuvant could be effective vaccine candidates against *staphylococcosis* disease in *Labeo rohita* fingerlings.

KEYWORDS: *Labeo rohita*, Whole cell, Outer membrane protein, *Staphylococcus aureus*, immunoadjuvant, *Andrographis paniculata*, mortality, relative percent survival.

INTRODUCTION

Labeo rohita (Rohu) is the most important and dominant species among the three Indian major carps in terms of fish culturing systems (Swapna *et al.*, 2010). Rohu is the most preferred species and widely cultured in freshwater aquaculture for its high consumer preference and fast growth rate (Anderson, 1992). Worldwide, carp production depends on the cultivation of rohu, as it contributes 45% of the total fish production. Rohu attains good market value for its taste and flesh and one of the main distributor in freshwater aquaculture (FAO, 2010).

Staphylococcus aureus is an opportunistic pathogen and has been identified as the most commonly occurring bacterial pathogens in fish farms (Dey *et al.*, 1995). In fishes, contamination of water aids to the occurrence of Staphylococcosis diseases and it is mainly exacerbated in spring and summer causing severe mortalities in fishes (Varvarigos, 2001). Bacterial diseases in fish are the most important challenges that hamper the fish production and decline the country's economy.

Andrographis paniculata also known as 'Nilavembu', is most popular and being used for centuries as a medicinal herb with broad range of pharmacological activities (Misra *et al.*, 1992). "Andrographolide" is the key component of this plant, responsible for stimulating the immune system and commonly referred as "Immune booster" and "Anti-staphylococci" (Guy, 2007). The present study was aimed to develop whole cell and outer membrane protein vaccine along with immunoadjuvant *Andrographis paniculata* and to study the efficacy of the prepared vaccines when challenged against virulent *Staphylococcus aureus* in *Labeo rohita* fingerlings.

MATERIALS AND METHODS: EXPERIMENTAL FISH

Fingerlings of average weight 5g-10g were procured from Poondi fish farm in Thiruvallur. Fingerlings were acclimatized for about 15 days to meet the laboratory condition and water was renewed thrice in a week. Fingerlings were fed with ground nut oil cake once in a day. Water quality parameters such as pH, temperature and salinity were checked periodically.

Bacterial Strain

MTCC 2940 *Staphylococcus aureus* sub-culture was obtained from King Institute of Preventive Medicine and Research, Guindy.

Preparation of Bacterial Culture

Bacterial culture was grown in conical flask containing tryptic soy broth (TSB) medium at the temperature of 25^0C. Culture were maintained at -80^0C and the cell suspensions was maintained and stored in tryptic soy broth containing 25%(v/v) glycerol for further use (Fig-1).

Fig. 1: Inoculation of Bacterial Culture (*Staphylococcus Aureus*).

Preparation of Whole Cell (WC) Vaccine (Formalin-Killed)

Whole cell vaccine was prepared using the bacterial isolate *Staphylococcus aureus* in tryptic soy broth (TSB) for 24 hours and incubated at 37°C. Formalin (40% w/v) was added to the broth culture at a final concentration of 0.5% (V/V) and left for up-to 48 hrs in room temperature. The inactivated cells were then harvested by centrifugation at 4000xg for about 10 minutes. The prepared whole cell vaccine was then tested for their sterility (free from the living cells) by streaking them onto trypticase soy agar which showed no growth (Fig-2).

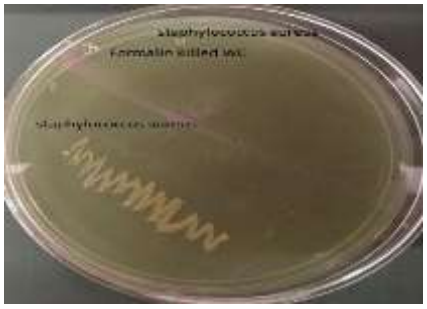

Fig. 2: Formalin Killed WC Vaccine.

Preparation of Outer Membrane Protein (OMP) Vaccine

Outer membrane protein (OMP) was prepared following the protocol of Austin & Rodgers (1981) with little modification. Bacterial culture, *Staphylococcus aureus* was grown in TSB (tryptic soy broth) at 37^0C for 24hours. The cells were harvested by centrifugation at 8500 rpm for about 10 minutes. After centrifugation, supernatant was discarded, and pellet was washed with 20mM Tris buffer at pH 7.2 and again the pellet was resuspended in 10mM EDTA buffer. Ultracentrifugation of collected supernatant was carried out at a speed of 27,400 rpm for about 45 minutes. The sediment collected after ultracentrifugation was OMP which was kept in -20ºC for further use.

Preparation of Immunoadjuvant-*Andrographis Paniculata*

Andrographis paniculata (Nilavembu) plant powder were purchased from Sri Jayendra Saraswathi Ayurveda College and Hospital, Poonamalle, Chennai-600123. Plant powder were extracted with hot water at 100^0C for 2hours using Soxhlet method. Plant extracts were filtered thoroughly, and the supernatants were condensed using a rotary evaporator at the temperature of 55^0C, then it was lyophilised using a lyophilizer and stored at 4^0C for further use.

Qualitative Analysis of Proteins

Qualitative analysis of proteins for the prepared whole cell and outer membrane protein vaccines were performed using SDS-PAGE (Sodium Dodecyl Sulphate-Polyacrylamide gel electrophoresis) given by Laemmli in 1970 (Fig-3).

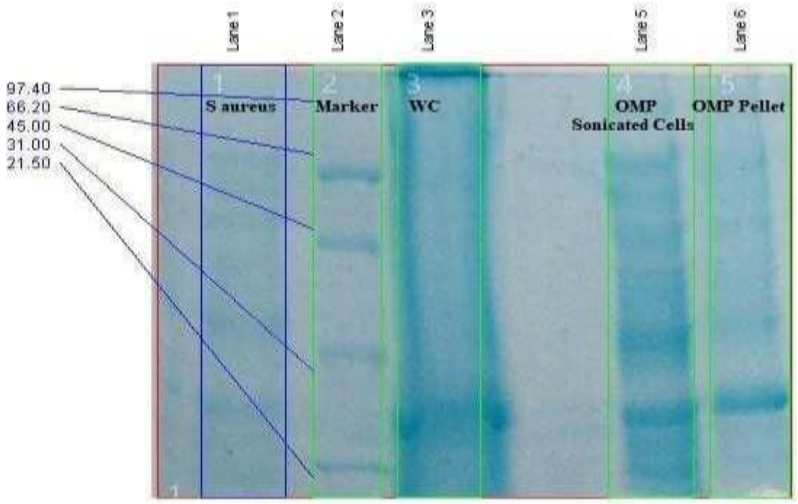

Fig. 3: SDS-PAGE Profile of WC & OMP vaccines with Molecular Weights (KDa).

Quantitative Analysis of Proteins

Total protein concentration of prepared whole cell and outer membrane protein vaccine was estimated using Lowry's Method (Fig-4).

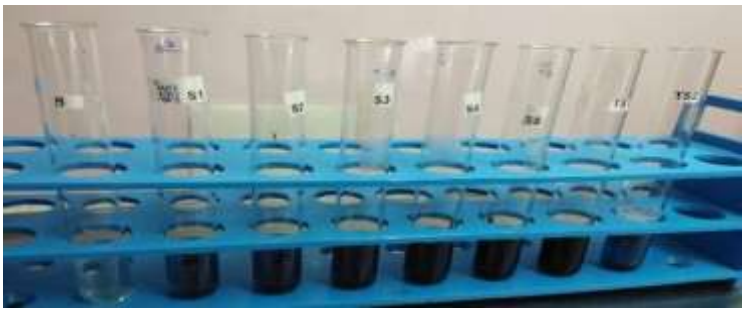

Fig. 4: Protein Estimation of WC & OMP.

Vaccine Delivery

25 Fingerlings were divided into six groups of (blank, control, WC, WC+A, OMP, OMP+A) and feeding was stopped 24hours prior to the experimental day to respond better for the immunisation. The vaccine dose concentration was $1x10^8$ cells/ml. Fingerlings were immersed in hyper-osmotic solution of 2% Nacl for 5 minutes followed by the bath vaccination of about 30 minutes. Booster vaccine was given on day1 and second dose was given on day 30 to all the experimental groups (Fig-5).

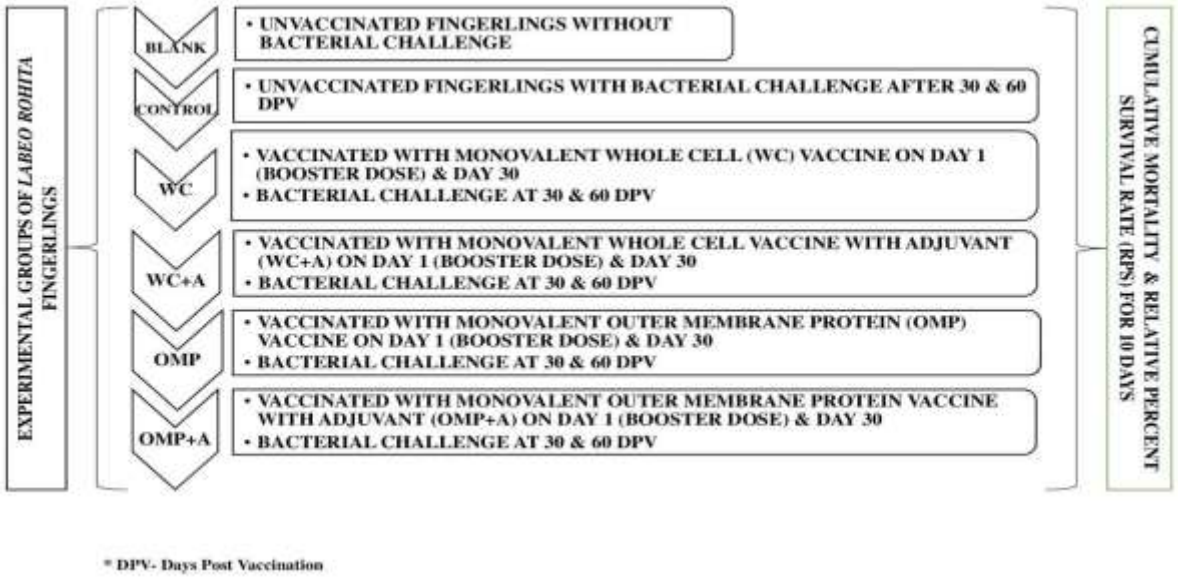

Fig. 5: Experimental Design of *Labeo Rohita* Fingerlings.

Challenge with *Staphylococcus Aureus*

After 30 and 60dpv fingerlings were bath challenged with virulent *Staphylococcus aureus*, in lethal dose of $1x10^8$ cells/ml. Challenge persists up-to 1 hour to all the experimental groups. Mortality and pathological symptoms were recorded for 10 days post-challenge and relative percent survival (RPS) was calculated using the formula given by Amend (1981),

$$RPS = 1 - \frac{\% \text{ of mortality in vaccinated groups}}{\% \text{ of mortality in unvaccinated groups}} \times 100$$

RESULTS AND DISCUSSION

Total protein concentration of whole cell vaccine was found to be 34 µg/ml and 22 µg/ml in outer membrane protein vaccine. SDS-PAGE results showed the, whole cell vaccine with a band length of 27.95 KDa and outer membrane protein vaccine with a band length of 27.43-35.43 KDa respectively.

After 30 dpv, mortality rate was 100% in control group and relatively low mortality was found in WC with 32% and 36% in OMP respectively (Table-1; Fig-6,7). While immunoadjuvant treated groups significantly reduced the mortality by 20% in WC+A and 28% in OMP+A groups. The above studies are similar to the studies conducted by Sahu *et al.*, (2007) in their studies, they found that, *Labeo rohita* fingerlings when vaccinated with monovalent *Aeromonas hydrophila* significantly reduced the mortality after 30 dpv and elevated the RPS rate up-to 70% when compared to control. After 30 dpv, significant increase in RPS was observed in WC with 80%, 72% in OMP respectively.

After 60 dpv, booster dose significantly increased the survival rate with 76% in WC, 88% in WC+A and 84% in OMP, 92% in OMP+A respectively (Table-2; Fig-6,7). As the relative percent survival increases, mortality rate was significantly reduced to 24% in WC, 12% in WC+A and 16% in OMP, 8% in OMP+A respectively. Anbarasu *et al.*, (1998) studied that, formalin inactivated vaccines provide sufficient protection when mixed with immunoadjuvant.

Thangaviji *et al.*, (2012) reported that, herbal immunoadjuvant *Asparagus racemosus* significantly improved the immunogenicity of antigens even in a low dose concentration. OMP vaccines with adjuvant, decrease the cumulative mortality to 30% in adjuvanted groups and 20% in non-adjuvanted groups, when challenged against *Aeromonas hydrophila* in goldfish (*Carassius auratus*).

Table 1: Mortality and Relative Percent Survival of *Labeo Rohita* Fingerlings After 30 DPV.

	TYPES OF VACCINES	METHOD OF VACCINATION	METHOD OF BACTERIAL CHALLENGE	NO. OF FISH	MORTALITY NO	(%)	RPS (%)
1	Blank	-	-	25	-	-	100
2	Control	-	Bath challenge	25	25	100	0
3	Whole cell (WC)	Immersion (Bath)	Bath challenge	25	8	32	68
4	Whole cell + Adjuvant (WC+A)	Immersion (Bath)	Bath challenge	25	5	20	80
5	Outer membrane Protein (OMP)	Immersion (Bath)	Bath challenge	25	9	36	64
6	Outer membrane Protein+Adjuvant (OMP+A)	Immersion (Bath)	Bath challenge	25	7	28	72

Table 2: Mortality and Relative Percent Survival Rate (RPS) of *Labeo Rohita* Fingerlings, After 60 DPV.

	TYPES OF VACCINES	METHOD OF VACCINATION	METHOD OF BACTERIAL CHALLENGE	NO. OF FISH	MORTALITY NO	(%)	RPS (%)
1	Blank	-	-	25	-	-	100
2	Control	-	Bath challenge	25	25	100	0
3	Whole cell (WC)	Immersion (Bath)	Bath challenge	25	6	24	76
4	Whole cell + Adjuvant (WC+A)	Immersion (Bath)	Bath challenge	25	3	12	88
5	Outer membrane Protein (OMP)	Immersion (Bath)	Bath challenge	25	4	16	84
6	Outer membrane Protein+Adjuvant (OMP+A)	Immersion (Bath)	Bath challenge	25	2	8	92

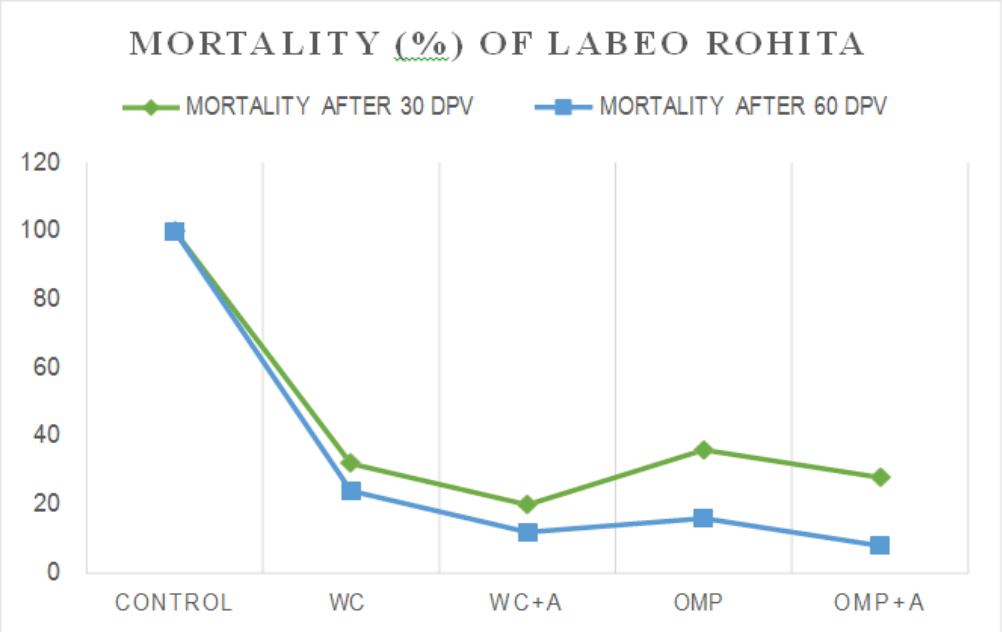

Fig. 6: Mortality of *Labeo Rohita* Fingerlings After 30&60 DPV.

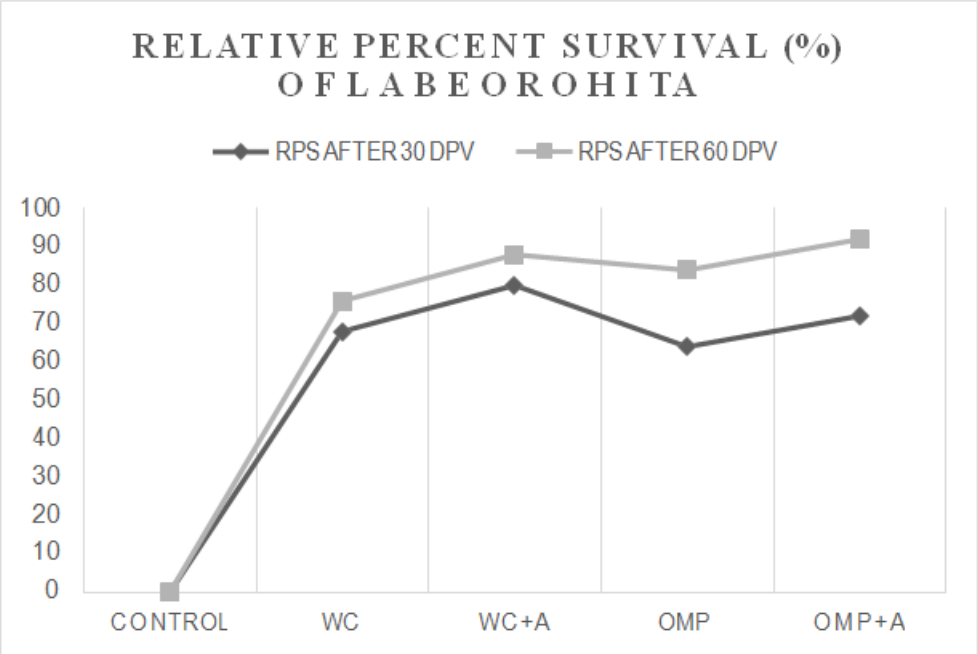

Fig. 7: Relative Percent Survival Rate (RPS) of *Labeo Rohita* Fingerlings, After 30&60 DPV.

CONCLUSION

Vaccines that are derived from the whole cell and outer membrane protein of bacteria provides long-term protection. With tremendous increase in freshwater aquaculture, the need for effective vaccine to control bacterial diseases is of prime concern. Immersion vaccination is gaining importance in aquaculture for its cost-effectiveness and one of the widely used techniques in aquaculture industries. To conclude, whole cell and outer membrane protein vaccines with and without adjuvant provided sufficient protection to the *Labeo rohita* fingerlings when challenged against *Staphylococcus aureus.* Hence, the prepared WC and OMP vaccines could be an ideal vaccine candidate to control Staphylococcosis disease in aquaculture practices.

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