Full Length Article

A REPORT ON HERBAL REMEDY OF CONJUNCTIVITIS OF RURAL POULTRY: RARE CASES OF *SALMONELLA SPP.* OF OCULAR INFECTION

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ABSTRACT

In vivo study was carried out to study the therapeutic efficacy of Nicobarese ethno veterinary medicinal practice for eye infection in rural poultry prepared from herbal plants. In house herbal formulation was prepared using extracts of Glycosmis pentaphylla and Spondias pinnata and its antibacterial sensitivity against Salmonella spp. isolate from ocular infection of rural poultry. It was reported that significant effect of the plant extract on the isolated bacteria. Hence, it can be inferred that the herbal formulation can serve as an alternative to quinolone group of antibiotics for the treatment of conjunctivitis in rural poultry production

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INTRODUCTION

Bilateral localized conjunctivitis is one of the common manifestations in poultry species that leads to severe morbidity and economic losses and is caused by bacterial origin, viral diseases (*Infectious Coryza, Ranikhet disease, Infectious Laryngotracheitis*, Swollen head syndrome (avian meta-pneumovirus), fungal origin and parasites (*Oxyspiruramansoni, Thelazia spp., Ceratospira spp., Plasmodium spp., Microsporidiosis, Cryptosporidial* or *trichomoniasis* and *Aspergillus fumigates* (Doneley et al., 2013). Besides, secondary manifestation of periorbital or orbital diseases is particularly chronic rhinitis, sinusitis, septicemiasis and bilateral localised conjunctivitis in avian species (Williams 2013). The conjunctivitis is diagnosed by presence of reddened and inflamed cornea and conjunctiva of the eye and its discharge which crusting over eyelid and formation of pus if left untreated and ultimately terminates in vision loss (Doneley *et al*., 2013). Eye

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infections have significant economic value because they adversely affect the health and performance of the birds. The conjunctivitis is also frequently found among rural and Vanaraja poultry birds in Andaman and Nicobar Islands where more than 60% of poultry population belong to backyard system which underlines the importance of treatment for this disease condition (Evans *et al*., 1955, Laursen-Jones, 1968). Topical antibiotic and antifungal agents are commonly used to treat conjunctivitis with higher cost involvement. Further, continuous uncontrolled use of antibiotics in-turn causes development of antibiotic resistance. Moreover, the surveillance data (Greene *et al*., 2008, Dalhoff, 2012) indicated that resistance of *Salmonella* serovars is consistently highest for antimicrobial agents (Quinolones) that have been used for longer time period in veterinary medicine to treat the diseases in poultry.

To diagnose the cases of conjunctivitis, the present study attempted for isolation of bacteria which is ultimately causes secondary infection subsequent to primary causes. Ethno-veterinary medicine (EVM) system, an indigenous knowledge on animal health is re-emerging as holistic animal health care approach. Number of indigenous practices in the form of decoction, pulp, ashes and crude juice of medicinal plants has been reported among rural community to cure conjunctivitis (Ali and Shankar 2014). Considering the economic importance of conjunctivitis in rural poultry of Andaman and Nicobar Islands and significance of re-emerging field of ethno veterinary medicine and after successful isolation of the causative organism we carried outin vivo study to explore the therapeutic efficacy of ethno veterinary medicinal plants for conjunctivitis in rural poultry prepared from herbal plants which are endemic to this Island.

METHODOLOGY

Collection of samples, isolation of organisms and antibiogram

A total of 120 eye swabs were collected from conjunctivitis affected birds from villages viz., Chouldhari (11.6405° N, 92.6611° E), Rangachang (11.5840° N, 92.7350° E) and Siphighat (11.48326° N, 92.6077°) of South Andaman. The ailing birds showed clinical signs of having swollen and closed eyes with pus. Collected swabs were transported to the laboratory as per the standard methods for bacteria isolation (Cheesbrough, 2006; Winn *et al*., 2006). The swabs were in oculated in nutrient broth and incubated at 37°C for overnight (12 hours). The broth culture was streaked on next day in MacConkey plates and incubated at 37°C for overnight (12 hours). The colonies of isolates were identified as *Salmonella spp*. based on colonial morphology and biochemical tests (HiMedia, Mumbai, India) as per standard methods (Quinn *et al*., 2002, Cappuccino and Sherman, 2001, Cheesebrough, 2006).

Antibacterial sensitivity tests were performed on *Salmonella* isolates (n=35) by single-disk diffusion method (Bauer *et al*., 1966) in accordance with National Committee for Clinical Laboratory Standards (NCCLS, 1993). A total of 10 antibiotic discs (Gentamicin (10µg), Penicillin (10µg), Chloramphenicol (30µg), Ampicillin(10µg),

Trimethoprim (10µg), Tetracycline (30µg), Erythromycin (15µg), Enrofloxacin (10µg), Ofloxacin (5µg) and Ciprofloxacin (10µg) were assayed. The diameter of the zone of inhibition (ZI; mm) was measured. The antimicrobial susceptibility data was expressed as percentage of the isolates. Multi-drug resistance was defined as resistance exhibited to three or more antimicrobials (Tricia *et al*., 2006).

Collection of herbal plant, processing for extraction, antibiogram study with conventional antibiotics and herbal extract

A total of two ethno medicinal plants (Plate 1) were selected for the present study based on participatory survey with tribal farming community. The collection was made on the basis of traditional knowledge of Nicobarese tribal community settled in villages like Arong (9.1615° N, 92.7518° E), Mus9.2415° N, 92.7817° E) and Harminder Bay (N 92°32.005' E). First two villages are situated in Car Nicobar group of Islands and last one is situated in Little Andamanas these plants are commonly used for various ailments in particular eye infections by the Nicobari tribes. Phytochemical of study of the plants were also carried out to quantify phenolics, flavonoids and carotenoids following standard methodology (Sulaiman and Balachandran 2012).

These medicinal plants were processed for methanolic extraction. Collected samples were washed with tap water, shade dried and powdered. These dried samples were soaked in methanolat ratio of 1:10 (w/v) for 3 days. After three days, it was filtered and kept at 40 – 50°C in water bath for 6 to 8 hours until methanol is completely evaporated. Subsequently, methanol extracts of dried leaf samples dissolved in 10% v/v dimethyl sulfoxide (DMSO) at the ratio of 1:5 (Mishra and Padhy, 2013). The bacterial strains were screened for susceptibility/ resistant to different medicinal plant extracts with the dilution rate of 10-7 by use of the Kirby-Bauer's disc diffusion method using Mueller–Hinton (MH) agar medium (HiMedia, Mumbai, India). Six mm filter paper discs were prepared and autoclaved (121°C and 15lb pressure) which were soaked with the extracts (10-90mg). A Gentamicin (G-10µg) disc was used as a standard antibiotic and DMSO was added in a separate disc. The plates were incubated for 24 h at 37°C and after incubation the ZI was noted against each extract (Growther and Sukirtha, 2018). Herbal antibiogram of EVM plants against isolates was recorded using ZI (mm).

In house herbal formulation

Extract was prepared from dried and powdered leaves of *Glycosmispentaphylla and Spondiaspinnatain* coconut and castor oil as carrier at appropriate proportions and filtered hygienically to get clear drops extract. Sensitivity or resistant pattern was defined following standard recommendation (below 12 mm of ZI speaks for resistant bacterial population). In brief, data are presented as mean of measurement of zone of inhibition (IZ) of three replicates measured in mm. IZ 0-6 mm: no activity; 7-10 mm: weak inhibition; 11-15 mm: moderate inhibition;

more than 16 mm: strongly inhibited (Harun *et al*., 2016).

Data were expressed as mean ± S.E.M. Statistical reading and comparison among the groups was performed by one way analysis of variance (ANOVA)by least significant differences (LSD) test with a p value ≤ 0.05 was considered significant (Bhandary *et al*., 2012).

In vivo study of herbal extract in Vanarajareared under free range rearing system

A total of 75 ailing birds of South Andaman district were considered for this study. The birds were divided into three groups and (25 birds in each group). Group I: was treated with commercially available gentamycin eye drop, Group II: was treated with commercially available chloramphenicol eye drop, and Group III: was treated with in house herbal formulation. Observation was taken at 48 h intervals till recovery of the birds.

RESULTS AND DISCUSSION

The phytochemical content of both the plants has been depicted in Table-1.

Out of 120 samples, 70% of isolates were identified as Salmonella spp. based on their colony characteristics and biochemical profile.

The present study with clinical conjunctivitis of poultry due to isolate of Salmonella sps. is confirmed by reports (Mario Padron, 1990,) where Salmonella sps.was the primary cause for the blindness and conjunctivitis. The isolation rate of organism was found more in eye swab from clinical conjunctivitis than the rectal swab (Bhuvaneswari *et al*., 2015) and other resources such as feed, drinking water and egg samples (Samanta *et al*., 2014, Xavier *et al.*, 2011, Okonko *et al.*, 2010, Kilonzo-Nthenge *et al*., 2008).

However, this higher rate of isolation of salmonella sps.from eye infection is definitely possessing a risk to poultry industry and considered to be a severe public health hazard.

This is awell known fact that poultry serves as main reservoir of non typhoidal *Salmonella* (NTS). The mostly desperate species under this category are *Salmonella typhimurium*, *S. enteritidis, S. heidelberg* and *S. new port*. (Shaji *et a*l., 2023). Out of four species, *S. typhimurium* and *S. enteritidis ar*e responsible for most of the poultry related food borne illness (17.4 and 34% respectively) (Painter *et al*., 2013). Both these species colonize in the caecum (Tellez *et al*., 2001). Therefore, we presume this species of *Salmonella* is having the affinity to produce the ocular lesion. There are very few reports in the past that *S. arzonae* could be isolated from eye and brain lesions of broiler birds and turkeys (Silva *et al.*, 1980; Lavicka and Matusi, 1982).

Phenotypic detection of Antibiotic resistance

The antibiotics profile of *Salmonella* isolates were determined using specific

antibiotic discs (Table 2). All the confirmed *Salmonella* isolates showed absolute resistance to penicillin, trimethoprim, tetracycline and ampicillin whereas 75% of isolates showed resistance to erythromycin. The next higher incidence of resistance among the isolates was to chloramphenicol. All these isolates were highly susceptible to gentamicin. Partial resistance was observed for chloramphenicol (20%), enrofloxacin and ofloxacin (30%) and ciprofloxacin (40%).

On the contrary inhouse preparation of herbal extracts showed 16.05 mm zone of inhibition. From this observation, we could conclude that the lsolated organism is sensitive to in-house herbal preparation with *Spondias pinnata* and *Glycosmis pentaphylla*.

Clinically before the treatment, the ailing birds showed the symptoms of kerato-conjunctivitis, purulent eye discharge and swelling of eye lids (Plate 3). After 7 days application of medicine, there was partial recovery of ailing birds after treatment with Gentamicin and in house preparation of herbal medicine (Plate 4).

The lesion of partial recovery was reduction of purulent discharge and redness after treatment with Gentamicin and phytobiotics. After 9th day post treatment with Gentamicin and herbal preparation, there was complete recovery of ailing birds (Plate 5). On the contrary, in the Chloramphenicol treated groups, there was no clinical improvement in the ailing birds.

Multidrug resistant against commonly used antibiotics is common characteristic of *Salmonella spp*. and reported very frequently from different parts of world. To mention a few, is resistance towards penicillin, tetracycline and chloramphenicol which may by attributed towards sub therapeutic use of antibiotics. Earlier reports suggest that resistant *Salmonella* serotypes have been identified from most parts of globe against quinolones, chloramphenicol and cephalosporin (Karon *et al*., 2007; Eckert *et al*., 2010, Marshall and Levy, 2011; Shaji *et al.*, 2023).

Prebiotic (Boguslawska – Tryk *et al*., 2012), probiotic (Oh *et al*., 2017), symbiotic, postbiotic (Humam *et al*., 2019) and phytobiotic (Laptev *et al*., 2019) are used to mitigate the antibiotic resistance of such organisms.

In the present study, we could find that in-vivo application of gentamicin and our in house preparation of phytobiotics has shown 100% clinical improvement of the infected birds. Further, the result showed that significant effect of the plant extract on the isolated bacteria indicated that it can serve as an alternative to quinolone group of antibiotics for the treatment of conjunctivitis in rural poultry production.

In future study, speciation of organisms will be taken up since in this present communication we want to convey an interesting finding of herbal product effective *in-vitro* and *in-vivo* against antibiotic resistance *Salmonella spp.*

CONCLUSION

The scientific information in the present study is an addition to knowledge pool about efficiency of endemic plants of Andaman and Nicobar Islands (*Spondias pinnata* and *Glycosmis pentaphylla*) against kerato-conjunctivitis of rural poultry. This observation has scientifically authenticated the traditional knowledge of Nicobari tribes about efficacy of these plants against ocular infection. Therefore, it is important to conduct the surveillance programmes on antibiotic resistance in clinical conjunctivitis in both veterinary and human medicine. This practice can also go a long way to minimize the emergence of resistance bacterial strains in human and animals.

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Table.1. Phytochemical content of both the medicinal plants

Plants	Total phenolics (mg/100g)	Total flavanoids (mg/g)	Total carotenoids (µg/g)
Glycosmis pentaphylla	2552.5	2.55	23.38
Spondias pinnata	907.5	0.91	18.25

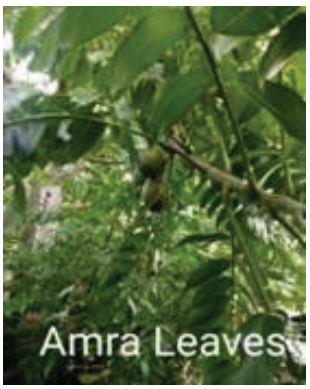
Table.2. Multi drug resistant pattern of the *Salmonella spp*. isolated from poultry conjunctivitis

Antimicrobial agents ((µg/disc)	Isolates (n=35)	
	% Resistant	% susceptible
Gentamicin (10µg)		100
Penicillin (10µg)	100	-
Trimethoprim (10µg)	100	-
Tetracyclin (30 µg)	100	-
Ampicillin(10µg)	100	-
Erythromycin(10µg)	75	25
Chloramphenicol(30µg)	50	50
Enrofloxacin(10µg)	50	50
Ofloxacin (5µg)	30	70
Ciprofloxacin(10µg)	40	60

Table.3. Antibacterial activity as size of inhibition zone of methanolic extracts of leaves of ethno medicinal plants of Nicobari tribal farming community against *Salmonella typhimurium* isolates of clinical conjunctivitis

Sl.No	Medicinal plants	Zone of Inhibition (mm) NS
1	*Spondias pinnata*	15.6±0.23
2	*Glycosmis pentaphylla*	15.32±0.31
3	*Phytobiotic formulation*	16.21±0.51

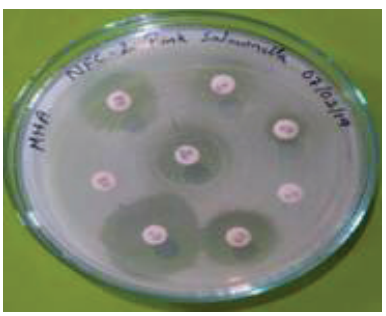
Plate.1. Ethno Medicinal plants of Nicobar tribal farming community



Amra (*Spondias pinnata***)**

Raneúl (*Glycosmis pentaphylla***)**

Plate.2. Antimicrobial susceptibility of Salmonella isolates of clinical conjunctivitis towards antibiotics and extracts of leaves of ethno medicinal plants of Nicobari tribal farming community.



Gentamicin

Glycosmis pentaphylla and *Spondias pinnata*

Plate.3. Kerato conjunctivitis case

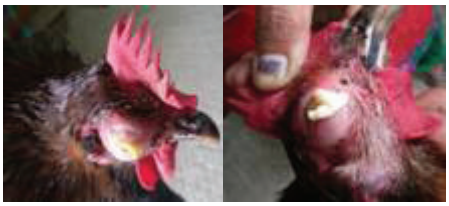
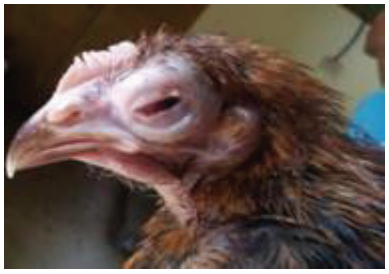


Plate.4. Partial healing with in house preparation

Plate.5. completely recovery with in house preparation



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