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Full Length Research Paper

Efficacy of resinous extract from C*ommiphora swynnertonii (Burrt)* against Newcastle infection in chickens

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Antiviral effect of resinous extract from *Commiphora swynnertonii* was investigated using chickens experimentally infected with Newcastle disease (ND) virus. A total of 114 chickens, approx. 5 months old, were assigned into eight experimental groups (G1 – G8). Chickens in G1 and G2 served as negative (not infected, not treated) and positive (infected, not treated) controls. To study the prophylactic effect of the extract, chickens in G3, G4 and G5 received 250, 500 and 1,000 mg resin per kg bodyweight respectively seven days before experimental infection. Chickens in G6, G7 and G8 received 250, 500 and 1,000 mg resin per kg bodyweight respectively seven days after infection with ND virus to assess therapeutic effect of the extract. Clinical signs, bodyweight gain and mortality were monitored. Antibody titers against the virus were measured, postmortem and histopathological lesions were examined. Results revealed significant reduction (P< 0.05) in clinical signs and mortality rates following administration of the resinous extract before and after the infection. Prophylactic administration of the extract was more effective in reducing the severity of the disease compared to the therapeutic approach. Similarly, antibody titers decreased significantly (P< 0.001) in all resin-treated groups irrespective of dose given and on whether the extract was administered before or after ND was evident. These findings indicate that the resinous extract from *C. swynnertonii* has strong antiviral activity against ND virus in chickens and that prophylactic administration has better protective effect against the disease.

Key words: *Commiphora swynnertonii,* Newcastle disease virus, resinous extract, chickens.

INTRODUCTION

Throughout history, plants have been used as a rich source of medicines and pharmaceuticals (Schmidt et al., 2008). Herbal preparations are frequently used in management of poultry diseases in rural areas (Bizimana, 1994; Gueye, 1999; ITDG and IIRR, 1996; Musa, 2008). Newcastle disease (ND) is a viral disease and one of the major constraints to the poultry industry especially in the developing countries including Tanzania (Yongolo, 1996). The disease, which affects only birds, causes serious economic loses because it is highly contagious with high mortality rates. Several researchers have attempted to manage ND using herbal preparations. Stems of *Euphorbia candelabrum*, (Euphorbiaceae) fruits of *Capsicum annum* (Solanaceae) and *Iboza multiflora* (Liliaceae) have been tested against ND in chickens (Waihenya et al., 2002, Mtambo et al., 1999). However, results from these studies did not show any significant antiviral effect against ND virus. In a recent study by Bakari et al., (2012), extract from the resin of *Commiphora swynnertonii* was shown to have a significant negative effect against ND virus in embryonated chicken eggs. Nevertheless, there are no reports on the effect of *C. swynnertonii* against ND virus in infected chickens. Therefore, the current study was carried out to investigate the effect of the resinous extract from *C. swynnertonii* in growing chickens experimentally infected with the ND virus.

Material and methods

Study area and test plant

This study was carried out at Sokoine University of

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Table 1 Treatments allocation.

Trial	*Groups*	*N*	*Treatment (resin mg/kg bodyweight)*
Control	G1*	12	0
	G2†	12	0
Prophylactic	G3	15	250
(treatment before infection)	G4	15	500
	G5	15	1,000
Therapeutic	G6	15	250
(treatment after infection)	G7	15	500
	G8	15	1,000

*Negative control - not infected; †positive control - infected

Agriculture in Morogoro Municipality. The test plant material (resin) was sourced from Simanjiro District in Manyara Region which is located 4°0′0″ S, 36°30′0″ E and 1,360 m a above sea level. The plant was identified by a botanist as *Commiphora swynnertonii* and a voucher specimen number CK 6489 was prepared and preserved at the National Herbarium in Arusha, J. Kayombo, personal communication, 2009. The resin was collected as exudates from stem bark incisions into wide mouthed glass bottles and transported to laboratory for extraction and testing.

Experimental animals

Hundred and fourteen (114) one-day old chicks (Black Australorp) were purchased from a single farmer in the study area. The chicks were raised in a large brooder and maintained on maize bran-based chick mash with *ad libitum* drinking water for two weeks and then they were dewormed and vaccinated against infectious bursal disease (IBD). After a month of brooding, the chicks were moved into rearing cages for four months before they were randomly assigned into different experimental groups. In the rearing cages, chicks were maintained on growers mash, given mineral supplements (Amintotal®) and *ad libitum* access to drinking water. At the age of 5 months, chickens were shifted to experimental cages, wing tagged and randomly assigned into eight groups (n = 12 – 15 chickens). Weekly bodyweight gains were measured and chickens were observed for any signs of diseases.

Preparation of NDV inoculums

ND viruses were isolated from livers and proventriculi of naturally infected chickens using a protocol described by Senne (1998) and modified by Sally (2002) and Waihenya et al., (2002). The organs were cut into small pieces and washed with phosphate buffer saline (PBS) before being ground into homogenious mixture using a mortar and pestle with aid of sterile sand. The resultant mixture was sterilized by diluting it with antibiotic medium (PBS with 200 IU/mL penicillin, 2 mg/mL, streptomycin, 0.05 mg/mL, gentamycin and 1,000 IU/mL nystatin at pH 7.0 - 7.4) to make 20% w/v suspension. Thereafter, the sterilized suspension was spinned in a chilled (4 - 10^0C) centrifuge at 1,000 – 1,500 rpm for 10 minutes to sediment tissue debris, bacteria and fungi. The supernatant was removed aseptically and incubated at room temperature for 90 minutes. To propagate the viruses, haemaglutination test was carried out and the inoculums containing 32 HA titres were inoculated in embryonated chicken eggs (ECE) through allantoic cavity. After four days, allantoic fluids were collected and serially diluted in PBS to obtain a dilution of 10^{-6} of viruses which was later used to induce ND in the experimental chickens.

Preparation of the extract

The resinous material was soaked in ethanol (99.8% v/v) and filtered (Whatman No. 1); the filtrate was concentrated immediately using a rotary evaporator. The resulting crude extract was then stored at 4°C in airtight bottles until used in the experiment.

Experimental design

Two concurrent trials were carried our in order to investigate both prophylactic and therapeutic effects of the resinous extract from *C. swynnertonii* against experimental ND infection in chickens. The eight chicken groups (G1 - G8) received different treatments as shown in table 1. Chickens in G1 were neither infected with ND virus nor treated with the extract (negative control) whereas those in G2 were infected but were not treated (positive control). Distilled water placebo was administered orally to chickens in the two control groups for 7 days.

Table 2 Clinical signs score scale.

Score	Clinical signs
1 - Normal	No disease signs (healthy chickens)
2 - Mild	Depression, anorexia (reduced feed intake)
3 - Moderate	Depression anorexia, greenish diarrhea
4 - Severe	All signs mention above together with difficult breathing (dyspnoea), raised body temperature (> 42^0C), loss of bodyweight emaciation and/or death

Table 3 Body condition scores and mortality rates of chickens with experimental ND following treatment using resinous extract from *C. swynnertonii*.

Trial	Group	Dose (resin mg/kg)	Body condition score	Chickens in score scale	Deaths	Mortality rate (%)
Control	G1	0	1	12	0	0.0
			2	0		
			3	0		
			4	0		
	G2	0	1	0	12	100
			2	3		
			3	4		
			4	8		
Prophylactic (treatment before infection)	G3	250	1	4	6	40.0
			2	3		
			3	1		
			4	0		
	G4	500	1	12	5	33.3
			2	3		
			3	1		
			4	1		
	G5	1,000	1	13	5	33.3
			2	2		
			3	1		
			4	0		
Therapeutic (treatment after infection)	G6	250	1	1	11	73.3
			2	3		
			3	3		
			4	8		
	G7	500	1	1	10	66.7
			2	3		
			3	7		
			4	5		
	G8	1,000	1	2	6	40.0
			2	4		
			3	7		
			4	3		

Chickens in G3 - G5 were used in the prophylactic trial and received oral doses of 250, 500 and 1,000 mg resin/kg bodyweight respectively for seven consecutive days before they were orally inoculated with 0.1 mL of viral suspension (containing 5.12 x 10^2 ND viruses per mL). Following appearance of ND clinical signs, chickens received another round of treatment with the resin as before for another seven consecutive days.

Table 4 Body weight (g) changes

Trial	Group	Dose	Days on treatment				
			Day -7	Day 0	Day 7	Day 14	Day 21
Control	G1	0	998	987.7	1,288.2	1,431.4	1,734.0
	G2	0	1,014	1,013.5	918.8	858.3	612.0
Prophylactic	G3	250	1,301	1,202.3	1,151.5	1,098.0	1,132.0
	G4	500	1,308	1,073.1	973.0	890.4	976.0
	G5	1,000	1,260	1,243.3	1,062.5	991.6	1,030.0
Therapeutic	G6	250	1,243	1,030.1	1,000.3	916.3	900.0
	G7	500	1,073	1,081.4	920.3	857.1	798.0
	G8	1,000	1,273	1,184.0	965.4	783.7	745.0

The therapeutic trial involved chickens in G6 – G8; these birds were first inoculated with 0.1 mL of the ND viral suspension and left for clinical sign of the disease to appear. Following appearance of clinical signs of ND, chickens in G6 – G8 were orally dosed with 250, 500 and 1,000 mg resin/kg bodyweight respectively for seven consecutive days.

Haemaglutination inhibition assay

Antibody titres against ND virus were estimated using haemaglutination inhibition assay (HI) as described by Sally, (2002). Blood samples were collected from five chickens selected randomly from each group and immediately centrifuged at 3,000 rpm for 10 minutes. Then, 25 µL of the harvested serum were serially diluted with 25 µL of PBS at pH 7.2 in microtitre plates. Antigen (25 µL of the 4HA dilution) was then added to each well before 50 µL of 1% red blood cells (fresh chicken erythrocytes) were added, mixed and allowed to incubate for 45 minutes at room temperature. After incubation, virus titres were read as the reciprocal of the highest dilution that caused agglutination of the chicken red blood cells.

Other observations

All chickens were weighed weekly and monitored daily for appearance and disappearance of clinical symptoms of ND. Clinical signs were assessed using a body condition score scale of 4 points in which 1 indicated normal healthy chickens and 4 indicated severely affected chickens as shown in Table 2.
Deaths were recorded and dead chickens were taken for post mortem examination. Tissues, namely, muscle, liver, kidney and intestine were taken from chickens, which died from each group and preserved for histopathology and microbial culture to find out whether there were any concurrent infections. The preserved tissues were processed for histopathological examination as described by Drury and Wallington, (1976).

Data analysis

The data were analyzed by one way ANOVA using Gen Stat package (2011). Means among groups were compared using Student's - t – test and $p < 0.05$ was considered significant.

RESULTS

Five days following inoculation of chickens with ND virus suspension, 50% (57/114) of all infected chickens showed most of the clinical symptoms typical of ND. By day 10 post-infection (p.i.) most of the chickens in G2 had severe clinical signs of the disease as shown in Table 3. These clinical signs were less obvious in chickens in the prophylactic trial (G3, G4 and G5). No clinical signs of ND observed in the negative control group (G1).
There was significant difference in mortality rates among the positive control, prophylactic and therapeutic trials. Administration of the extract before inoculation of chickens with NDV significantly ($P < 0.001$) reduced mortality rates from 40% (G3) to 33.3 % (G4 and G5) on day 7 post treatment. Mortality rates of chickens in therapeutic groups were reduced ($P < 0.05$) from 73.3% (G6) to 66.7% (G7) and 40 % (G8) as shown in Table 3.
Post-mortem examination of dead chickens from the positive control group (G2) revealed lesions typical of ND, namely, emaciation, facial and peri orbital oedema, conjunctivitis and whitish mucoid creamy caseous material in trachea. The proventriculi were severely swollen with button ring like reddish lesions. Histopathological findings included congested livers,

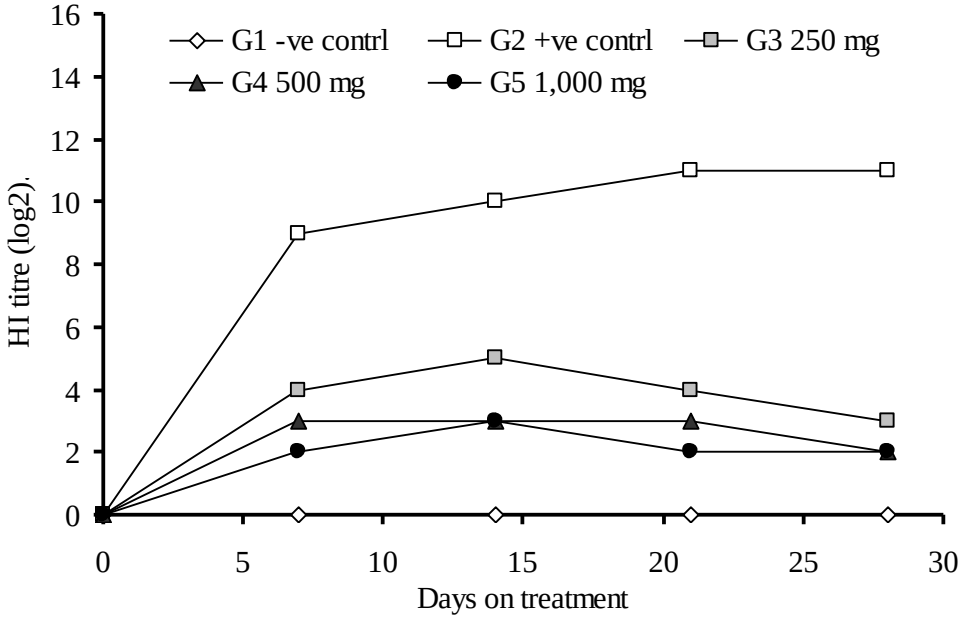

Figure 1. HI titre profiles of chickens infected with ND virus and treated with different levels of *C. swynnertonii* resinous extract before and after the infection (prophylactic trial).

lungs and intestines; perivascular cuffing with mononuclear cellular infiltrations were seen in tissues of all chickens infected with NDV. The lesions became less severe in all chickens which received the resin extract treatment regardless of the dose given.

Mean body weights of chickens in the prophylactic trial (G3, G4 and G5) decreased gradually with time until seven days after treatment before there was an increase towards 14 days after treatment was terminated (Table 4). In the therapeutic trial (G6, G7 and G8), the decrease in body weight with time was significant ($P < 0.01$). Furthermore, comparison of bodyweights on day 7 post treatment showed a decrease in bodyweight in a dose dependent manner ($R^2 = 0.98$; $P = 0.07$). The body weight of chickens in negative control group (G1) increased significantly ($P < 0.001$) to the end of experiment.

Antibody titres were detected starting from day 5 post infection in all infected groups. There was highly significant ($P < 0.001$) difference in levels of antibodies titres between positive control (G2) and the treated group irrespective of the dose of the extract given. The HI titres of chickens in G2 rose to a maximum of 2,048 by day 14 post-infection. However, in the prophylactic trial, results showed that administration of the extract reduced antibody titres in groups G3, G4 and G5 to a value of 8 (Figure 1). For the therapeutic trial (G6, G7 and G8), the antibodies titers decreased in a dose dependant manner ($R^2 = 0.79$; $P = 0.08$) from day 7 of treatment (Figure 2).

Negative control groups (G1) remained negative throughout the entire period of the experiment.

DISCUSSION

The current findings have demonstrated significant antiviral activity of crude resinous extracts of *C. swynnertonii* against experimental Newcastle infection in local chickens. The typical clinical signs which were observed following infection were a clear indication that the ND virus strain used was virulent.

Significant reduction in all clinical parameters, including mortality rates and pathological lesions of Newcastle infection suggested that crude resinous extract from *C. swynnertonii* had significant antiviral effect. Other *Commiphora* spp have been implicated in reducing severity of various viral infections in humans (Hanus et al., 2005) although the exact mode of action was not explained. Further indications of antiviral activity of the extract came from measurements of antibody titres against the ND virus during the chicken trials. Comparison of antibody titres between the two trials, showed that the levels of titres were significant lower in the prophylactic than in the therapeutic trial. This observation suggests that administration of the resin extract before the infection helped to reduce/interfere with virus multiplication with consequent reduced immunological response against the virus. Similar finding were observed when embryonated chicken egg were infected with ND virus and then treated with different

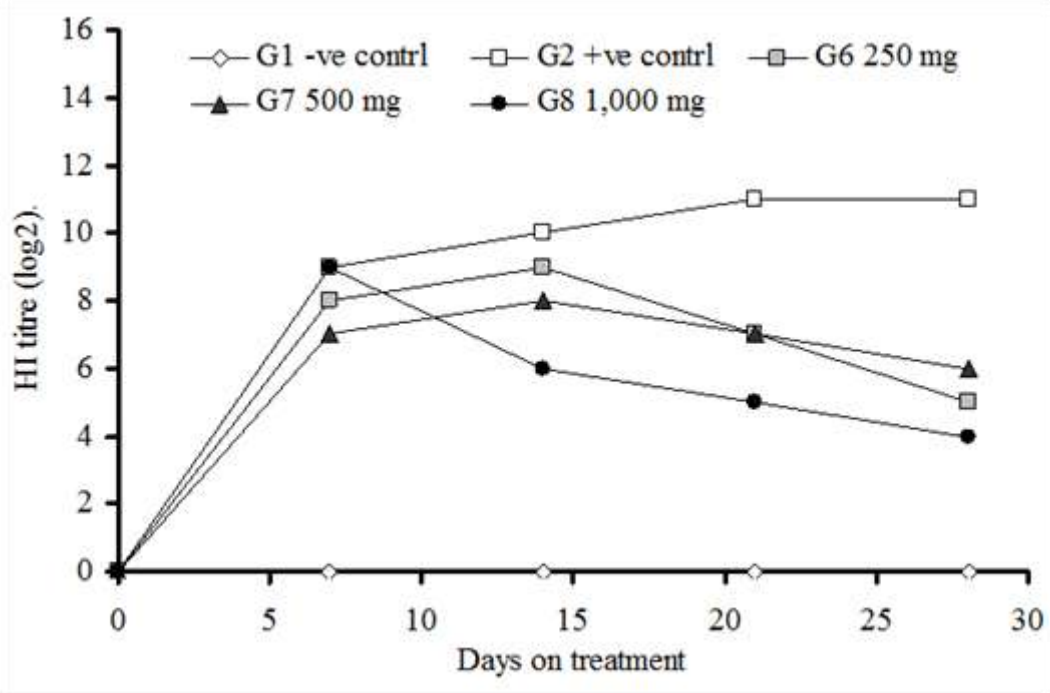

Figure 2. HI titre profiles of chickens infected with ND virus and treated with different levels of *C. swynnertonii* resinous extract after the infection (therapeutic trial).

extracts from *C. swynnertonii* (Bakari et al., 2012). Also, the reduction of antibody titres by the extract to the ratio of 1:8, which is regarded as protective antibody titre (Awan et al., 1994; Numan et al., 2005; Musa et al., 2009), is a further indication that the *C. swynnertonii* extract could be modulating the immune system of the chickens. Ezekiel et al. (2010) reported increased white blood cell counts following administration of *C. africana* in rats. Other studies (Mtambo et al., 1999; Waihenya et al., 2002) in which medicinal plants were tested against ND virus in chickens revealed that there were no significant differences in the levels of antibody titres between treated and untreated groups.

In the current study, it was also observed that an increase in the dose of the extract had a negative dose-dependent effect on bodyweight gain. This could be associated with the extract because some *Commiphora* spp have been used as anti-obesity remedy to reduce body weights in humans (Wang et al., 2004; Murray et al., 2006). The weight reduction effect has been associated with reduction in plasma cholesterol and glucose levels (Wang et al., 2004) through stimulation of thyroid hormone function thus interfering with basal metabolic rate leading to loss of body weight (Scott, 2005). Administration of *C. swynnertonii* resinous extract in healthy chickens significantly reduced concentrations of glucose and cholesterol in plasma (unpublished data).

The mechanisms through which the crude extracts from *C. swynnertonii* inhibit NDV multiplication in chicken's body are not yet known. However, many traditional medicinal plants used to treat viral diseases have been shown to contain high levels of compounds such as coumarins, flavonoids, alkaloids, terpenes, naphthoquinones and anthraquinones. Same classes of compounds have been found in *C. swynnertonii* and other *Commiphora* spp. (Hanus et al., 2005). These compounds exert their effect by killing the virus and/or interfering with viral multiplication (Jassim and Naji, 2003). Specifically, some of these compounds are speculated to exhibit protease inhibition, hence interferes with cleavage of haemaglutinin neuramidase and fusion protein, which are important glycoproteins for ND virus attachment and multiplication Zhirnov et al., (1985). Other classes of compounds such as flavonoids from *C. africana* have been reported to act by inhibiting production of prostaglandin (signaling molecule) and phosphosdiesterases involved in cell activation, the effect which predominantly depend upon biosynthesis of protein cytokines that mediate migration of circulating leucocytes to site of injury (Manthey et al., 2001) thus promoting healing.

In conclusion, findings from this study have demonstrated significant antiviral activity of resinous extract from *C. swynnertonii* against experimental ND infection in local chickens. Prophylactic administration of the extract could be a better approach in mitigating the effects of ND infection in endemic areas. Furthermore, therapeutic administration of resin extract after an outbreak could

also be used to reduce disease severity and mortalities. Field trials are recommended as a way of validating the use of *C. swynnertonii* extract against Newcastle disease in chickens.

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REFERENCES

Awan MA, Otte MJ, James, AD (1994). The epidemiology of Newcastle disease in rural poultry: A review, Avian Pathol., 23: 405-423.

Bakari GG, Max RA, Mdegela RH, Phiri ECJ, Mtambo MMA (2012). Antiviral activity of crude extracts from Commiphora swynnertonii (Burrt) against Newcastle disease virus in ovo. Trop. Anim. Health and Product. 44:7, 1389-1393.

Bizimana N (1994). Traditional Veterinary Practice in Africa. Schrifteib der GTZ. No. 243, Eschborn, Germany.

Drury RAB, Wallington EA (1976). Carleton's Histological Techniques, 4th Edition, Oxford University Press, London.

Ezekiel I, Mabrouk MA, Ayo JO (2010). Study on effect of hydro-ethanolic extract of *Commiphora africana* (Stem Bark) on inflammation and pain in rodents. Asian J. Med. Sci., 2: 81-84.

Guèye EF (1999). Ethnoveterinary medicine against poultry diseases in African villages. World's Poultry Science Association. World's Poultry Sci. J., 55: 187-194.

Hanus LO, Rezanka T, Dembitsky VM, Moussaieff A (2005). Myrrh-Commiphora, chemistry. Biomed. Paper, 149: 3–28.

ITDG and IIRR (1996). Ethnoveterinary medicine in Kenya. A field manual of traditional animal healthcare practices. Intermediate Technology Development Group (ITDG) and International Institute of Rural reconstruction (IIRR), Nairobi, Kenya.

Jassim SA, Naji MA (2003). Novel antiviral agents: a medicinal plant perspective. J. Appl. Microbiol., 95: 412–427.

Manthey JA, Grohmann K, Guthric N (2001). Biological properties of citrus flavonoids pertaining to cancer and inflammation. Current Med. Chem. 8: 135-153.

Mtambo MMA, Mushi E, Kinabo, LDB, Maeda-Machang'u A, Mwamengele LM, Yongolo MGS, Temu, RPC (1999). Evaluation of the efficacy of the crude extracts of *Capsicum frutescens*, *Citrus limoni* and *Opuntia vulgaris* against Newcastle disease in domestic a fowl in Tanzania. J. Ethnopharm., 68: 55–61.

Murray JK (2001). Coccidiosis in chickens. Agri-Facts. Practical Information for Alberta's Agriculture Industry Agdex 663-35.

Musa AA (2008). Antioxidant and antibacterial activity of *Commiphora kerstingii* (Engl.) stem bark extract. Res. J. Phytochem. 2: 106-111.

Musa U, Abdu PA, Dafwang II, Umoh JU, Sa'idu L, Mera UM, Edache JA (2009). Seroprevalence, seasonal occurrence and clinical manifestation of Newcastle disease in rural household chickens in Plateau State, Nigeria. Intl. J. Poult.Sci. 8: 200-204.

Numan M, Zahoor MA, Khan HA, Siddique M (2005). Serologic status of Newcastle disease in broilers and layers in Faisalabad and surrounding districts. Pakistan Vet. J. 25: 54-58.

Sally EG (2002). A basic laboratory manual for the small-scale production and testing of i-2 Newcastle disease vaccine. FAO Animal Production and Health Commission Asia and the Pacific 2002.

Schmidt B, Ribnicky DM, Poulev A, Logendra S, Cefalu WT, Raskin I (2008). A natural history of botanical therapeutics. Metab. Clin. Experim. 57: 3–9.

Scott AM (2005). Gum guggul and some of its steroidal constituents. Review of Toxicological Literature Prepared for National Toxicology Program (NTP) and National Institute of Environmental Health Sciences (NIEHS). National Institutes of Health U.S Department of Health and Human Services. Research Triangle Park, North Carolina, pp 18-21.

Senne DA (1998). Virus propagation in embryonating eggs, In: A laboratory manual for the isolation and identification of avian pathogens. David E. Swayne, John, R. Glisson, Mark, W. Jackwood James, E. Pearson and Willie M. Reed (eds), The Americans Associ. Avian Pathol. 235–240.

Waihenya RK, Mtambo MMA, Nkwengulila G (2002). Evaluation of the efficacy of the crude extract of Aloe secundiflora in chickens experimentally infected with Newcastle disease virus. J. Ethnopharm. 79: 299–304.

Wang XJ, Greiberger G, Ledinski G, Kager B, Paigenand G, Jurgen, (2004). The hypolipidemic natural product, *Commiphora mukul* and its component guggulsterone inhibit oxidative modification of LDL. Atherosc. 172: 239-249.

Yongolo MGS (1996). Epidemiology of Newcastle disease virus in village chickens in Tanzania. MSc Veterinary Medicine Dissertation, Sokoine University of Agriculture, Tanzania pp. 230.

Zhirnov OP, Ovcharenko AV, Burkriskaya AG (1985). Myxovirus replication of chicken embryos can be suppressed by Aprotonnin due to the blockage of viral glycoprotein cleavage. J. Gen. Virol. 66: 1633–1638.