COMPARATIVE EFFICACY OF NEGUVON AND NEEM (*Azadirachta indica*) LEAVES EXTRACT AGAINST STEPHANOFILARIASIS IN CATTLE IN DINAJPUR DISTRICT

A Thesis

By

MD. ANISUR RAHAMAN
Registration No: 1105124
Semester: July - December/2012
Session: 2011-2012

MASTER OF SCIENCE (M.S.)
IN
PHARMACOLOGY

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY,
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY (HSTU) DINAJPUR, BANGLADESH

DECEMBER, 2012

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Submitted to the Department of Physiology and Pharmacology

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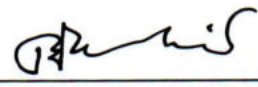
Approved as to style and content by-

Dr. Fahima Binthe Aziz
Assistant Professor
Department of Physiologyand Pharmacology, HSTU, Dinajpur-5200
Research Supervisor

Dr. Md. Nazrul Islam
Assistant Professor
Department of Pathology and Parasitology, HSTU, Dinajpur-5200
Research Co- Supervisor



Dr. Md. Bazlar Rashid
Assistant Professor
Chairman ofExamination committee
And
Department of Physiology and Pharmacology

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
FACULTY OF VETERINARY AND ANIMAL SCIENCE
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

DECEMBER, 2012

DEDICATED

TO
MY

PARENTS

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ABSTRACT

Stephanofilariasis one of the most problematic parasitic diseases caused by *Stephanofilaria assamensis* is endemic to Bangladesh. The disease could neither be fully controlled nor eradicated from these regions in spite of various attempts. The present study was conducted to find out the prevalence of the disease in the endemic areas, its economic impact and suitable measures for its eradication. The incidence rate was more in older animals. The direct and indirect economic losses to farmers by way of loss of draught days, delayed puberty, lower lactation yields and prolonged inter-calving period were enormous. The control and eradication of Stephanofilariasis in bovines could be successfully achieved especially by targeting the life cycle of the biological vector (*Musca conducens*), the intermediate host. Improvement in general cleanliness and hygiene to reduce the fly population. An experiment was carried out in nearby Villages and also in the Laboratory in the Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur during the period from July-December/ 2012. The Neguvon powder and the extract of Neem (*Azadirachta indica*) leaves were screened for assessment their comparative efficacy against humpsore in cattle. Among these, neem leaves extract were used topically to cover the affected area and Neguvon powder was applied topically twice daily to the cattle. 10gm Neguvon powder was mixed with 90gm Vaseline for 28days. Efficacy was recorded as compared to control. Extract of neem leaves showed gradual increase of efficacy up to day29th of treatment. Among two drugs Neguvon show better performance than neem leaves extract. But Neem leaves extract shows no side effect on body of cattle.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PHOTOGRAGH

LIST OF ABBREVIATION AND SYMBOLS

TEC	=	Total Erythrocyte Count
HB	=	Hemoglobin
PCV	=	Packed Cell Volume
ESR	=	Erythrocyte Sedimentation Rate
Bwt	=	Body weight
cm	=	Centimeter
Kg	=	Kilogram
gm	=	Gram
RBC	=	Red Blood Cell
Fig	=	Figure
No.	=	Number
mm	=	Mili mètre
hrs	=	Hours
DVHR	=	District veterinary hospital
%	=	Percentage
DNA	=	Deoxy Ribo Nucleic Acid
GR	=	Glutathion Reductage
Etc	=	Etcetera
GPX	=	Glutathion peroxidase
SOD	=	Superoxidase dismutase
CAT	=	Catalase
GST	=	Glutathion S-transferase
DTD	=	DT-diaphorase
HSTU	=	Hajee mohammad danesh science and technology university
DMBA	=	7,12,-dimethyl benz anthracene
BAU	=	Bangladesh Agriculture Universit

CHAPTER I

INTRODUCTION

CHAPTER I

INTRODUCTION

Stephanofilariasis in livestock are very common in Bangladesh. The *Stephanofilaria assamensis* entirely live on blood and lymph of all kinds of vertebrate animal. The damages caused by maggots are irritation, inflammation, exudation of lymph which coagulates to form crusts. *Stephanofilaria assamensis* cause dermatitis, alopecia and sometimes animals are infected secondarily by bacteria causing toxemia, septicemia and ultimately dead of the animals. (Chungsamarnyart, and Jansawan, 1991).

The present research is being conducted to study the efficacy of medicinal plant in veterinary practice. A large number of anthelmintics are now available in the market. The indiscriminate use of anthelmintics made the parasites to be resistant against the drug, which have been reported by experts throughout the world including Bangladesh (Hannan, *et al.,* 2001). So, we should have to back the traditional uses of me declines.

The Agro-ecological and geo-climatic condition of Bangladesh favors the survival, multiplication, spread and perpetuation of animal parasites. Moreover animal management and managerial system play an important role in high degree of occurrence of parasitic disease in Bangladesh. There are several indigenous medicinal plants (Mostofa, 1983) have anthelmintics action and used against both ecto and enodparasites in Bangladesh (Mostofa, 1983; Mannan, *et al.,* 1997) India (Dutta and Hazarika, 1976).

The contribution of livestock sub-sector is remarkable in Bangladesh. The contribution of this sub-sector to Gross Domestic Product (GDP) is approximately 6.5 percent (DLS1998) when the value of milk, meat, egg skin, fuel, draft, fertilizer etc. is considered. This sub-sector meets about 28 (Brammer *et al.*, 1996) of Gross Domestic Product (GDP). It provides full time employment to about

20% and generates 13% of total foreign currency and partial employment to about 50% of the rural population (Alam,1993).

Then in India the causal agent was discovered or recognized as *Stephanofilaria assamensis* (Pande, 1936). After that it was recognized in Malaysia as *Stephanofilaria Kaeli* (Buckley, 1939).

Stephanofilariasis is transmitted from one individual by fly that acts as an intermediate host of filarial worm. It has also been recognized that different types of vectors are responsible for different species of stephanofilaria. *Musca conducens* is identified as the vectors of *Stephanofilaria assamensis* in Bangladesh (Dewan and Rahman, 1970) and India (Patnaik and Roy, 1966, Patnaik, 1973).

Neguvon powder is very effective medicine against humpsore in cattle. It is found in market as trade name Neguvon (Bremer pharma, Germany). This product is used both as insecticide and parasiticide. It is Organophophorus compounds. Although it has basic property of inhibiting the action of cholinesterase. It is suitable for systemic action having high water solubility. Extract of neem leaves is used because it is easily available in our country and it has the antiseptic and also antiparacitic action. (Patnaik and Roy, 1966, Patnaik, 1973).

Considering all these facts in the present study Neguvon and Extract of Neem leaves were selected with a view:

1. To study the comparative efficacy of Neguvon and Neem leaves extract against humpsore in cattle.
2. To determine the effect of Neguvon and Extract of Neem leaves on some hematological parameters (TEC, ESR, PCV, Hb) in cattle.
3. To determine the effect of Neguvon and Extract of Neem leaves on some clinical parameters (severity of infestation, body weight, feeding efficiency, hair coat, and adverse effect) in cattle.

CHAPTER II

REVIEW OF LITERATURES

CHAPTER II

REVIEW OF LITERATURE

Bovine stephanofilarial dermatitis has been first reported from Indonesia (Ihle and Thle Landenber, 1933) popularly called "Cascado". Similar condition has been reported as verminous dermatitis in United States (Chit wood, 1934) and humpsore in India (Pande, 1936). It has been also reported from Japan, Africa, Denmark, Russia and Bangladesh. In the present study, available literature on Stephanofilariasis has been reviewed.

A skin disease caused by a filarial worm was first recorded by Dikmans (1934) in cattle in United States (North America) which was closely related to cascado of Indonesia. The lesions were found on the scrotum of two bulls.

Shemanchuk and Robertson (1990) stated that Stephanofilariasis or filarial dermatitisis present in cattle in southern Alberta, Canada. Lesions on the skin of cattle are caused by the nematode Stephanofilaria stilesi, which is transmitted by the horn fly (Haematobia irritans). Lesions resultining from a reaction to horn fly bites are similar in appearance to lesions caused by filariae and can be mistaken for filarial infestations. The effects of filarial infestations on livestock production appear to be minimal. A good horn fly control programme can reduce any damage that may occur as a result of the presence of the parasite.

Roy and Misra (1991) reported that of 35 exotic, cross-bred or indigenous cattle, aged 3-5 years, with typical lesions of hump sore on various parts of the body, 5 animals were left as untreated controls and the others, in groups of 10, were treated by topical application of 1% ivermtin ointment, 0.5% ivermectin ointment and 7.5% levamisole ointment daily for 4 weeks. The efficacy of the treatments, assessed after 6 months was 80, 50 and 50%, respectively.

Mostofa (1993) used an ointment Made of 1% Neguvon, 10% Neem leaves and barks, 89% Vaseline to treat humpsore of twenty five cattle. All the cattle were cured within 30 days by topical application of the ointment twice daily.

Baki (1995) recorded that 10% Neguvon ointment with 5% sulfanilamide powder and 5% zinc oxide was the most effective drug against humpsore as all animals had-both smaller and larger lesions were cured within 10-30 days. They higher effectiveness may be due to the use of zinc oxide which has the astringent action promoting healing of the lesions. A maximum of 30 gm of this ointment was required to cure the larger lesions.

Alam (1995) observed the therapeutic efficacy of Fenitrothion (Neguvon) and Neem seeds against stephanofilariasis in cattle. Neem seed kernel ointment and 10% Fenitrothion ointment were almost equally effective (100%) against stephanofilariasis in cattle. However, the recovery was faster (within 18-34 day) with 10% Fenitrothion ointment followed by Neem seed kernel ointment (27-42 days) and fresh Neem seed ointment (30-39 days). On the other hand, 5% Fenitrothion ointment was found almost ineffective against stephanofilariasis in cattle.

From this result, Neguvon and Asuntol were suggested as the most effective drug against humpsore. Encouraging results were also obtained after surgical removal of humpsore lesions.

Mannan (1997) reported that treatment of stephanofilariasis of group cattle treated with Ivomec $^{(R)}$ pour on at the dose rate of 500 mg/ kg, body weight topically on the sore showed slight improvement after 5th day of treatment which was assessed by reduction of itching, irritation and progressive drying. After application of 2nd dose at the 7th day there was significant improvement with reduction of the size of the lesions and at the end of the 18 post treatment days, Lesions were completely healed up by formation of smooth black scar tissue. It

was also observed that smaller lesions improved more rapidly than the larger lesion.

Rahim (1998) observed that subcutaneous preparation of ivermectin at the dose rate of 200 mg/ kg body weight against stephanofilarial dermatitis in both single and double dose of frequency was found effective without any side effect.

The edematous skin disease in buffaloes and cattle in Egypt caused probably by stephanofilaria had been treated with Furadan and Neguvon (R). Furadan was given at the dose rate of 50 ml/ animal intra muscularly in which 6-12 animals were improved. Five of 14 cutaneously at the dose rate of 12 ml/ 40 kg body weight and other 8 animals were cured ^{2nd} and 3^{rd} doses (Fouad *et al.*, 1974).

Gilmour *et al.* (1999) reported that fenitrothion is an organophosphorus insecticide widely used for the control of locusts in Australia and overseas. It is sprayed on swarms and bands of locusts and on the pastures on which they feed. However, there is little Australian data on depletion rates of fenitrothion when, used this not be able to, guarantee that their cattle meet legal requirements with respect to residue levels following grazing on sprayed pasture. This paper reports on a study conducted in February 1988 in central western New South Wales on the rate of depletion of fenitrothion in cattle, pasture and soil after fenitrothion spraying. The cattle were 18 month old heifers in forward store condition. Fenitrothion was detected in the fat of 7 of the 66 exposed cattle. Residues in 2 of these exceeded the Maximum Residue Limit of 0.05 mg/ kg. No fenitrothion was detected in the fat of the 26 cattle slaughtered 14 days or later after treatment or in muscle and liver samples. The half life of fenitrothion was 2-3 days in the soil and 1-2 days the pasture. It is concluded that the 14 days slaughter with holding period currently approved by the National Registration Authority is appropriate.

Humpsore due to stephanofilaria assamensis in Bangladesh recovered completely with Neguvon as 6% aqueous solution or 6% liniment in castor oil. In this trial one hundred and thirty six of 40 cattle were cured. The liniment proved superior

to aqueous solution. More than 40 days were required to cure the severe lesions. The drug was recommended as safe economic and highly effective. (Rahman and Khaleque, 1974).

Dasgupta *et al.* (2004) Numerous laboratory studies reveal that various naturally occurring dietary substances can modify the patho-physiological process of various metabolic disorders and can be an effective preventive strategy for various diseases, including cancer. Indian Neem tree, *Azadirachta indica* A. Juss. (family: Meliaceae), contains at least 35 biologically active principles and is widely grown all over the tropics. The effect of two different doses (250 and 500 mg per kilogram body weight) of 80% ethanolic extract of the leaves of Azadirachta indica were examined on drug metabolizing Phase-I and Phase-II enzymes, antioxidant enzymes, glutathione content, lactate dehydrogenase, and lipid peroxidation in the liver of 7-week-old Swiss albino mice. Also anticarcinogenic potential of Azadirachta indica leaf extract was studied adopting protocol of benzo(a) pyrene-induced fore-stomach and 7,12-dimethyl benz(a) anthracene (DMBA)-induced skin papillomagenesis. Our primary findings reveal its potential to induce only the Phase-II enzyme activity associated mainly with carcinogen detoxification in liver of mice. The hepatic glutathione S-transferase ($P < 0.005$) and DT-diaphorase specific activities ($P < 0.01$) were elevated above basal level. With reference to antioxidant enzymes the investigated doses were effective in increasing the hepatic glutathione reductase (GR), glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) activities significantly (from $P < 0.005$ to $P < 0.001$). Reduced glutathione measured as non-protein sulphydryl was found to be significantly elevated in liver ($P < 0.005$) and in extrahepatic organs (from $P < 0.005$ to $P < 0.001$) examined in our study. Glutathione S-transferase (GST) and DT-diaphorase (DTD) showed a dose-dependent increase in extrahepatic organs. Chemopreventive response was measured by the average number of papillomas per mouse, as well as percentage of tumor-bearing animals. There was a significant inhibition of tumor burden, in both the tumor model

system studied (from $P < 0.005$ to $P < 0.001$). Tumor incidence was also reduced by both the doses of *Azadirachta indica* extract.

Phukan *et al.* (2005) An epidemiological study on humpsore in cattle in Assam caused by Stephanofilaria assamensis **Pande, 1936** conducted from March 1999 to February 2000 revealed that out of 1189 cattle, 523 (43.98%) were positive for humpsore. Highest infection was observed in the monsoon season (53.43%) followed by post-monsoon (43.16%), pre-monsoon (33.54%) and lowest in winter (26.67%). Males (46.36%) and crossbred animals (50.41%) had a higher infection rate than the females (38.35%) and native cattle (41.14%). Animals below one year of age were free from infection, while animals aged between three to < six years had highest infection rate (57.08%). The infection rate was inversely proportional to the age of the animal. The study revealed that out of the 622 flies collected from the lesions, 305 were *Musca pattoni*, 199 *M. domestica* and 118 belonged to the subfamily Limnophaginae. Out of these three, only M. pattoni was found to harbour the larval stage of *S. assamensis*. The highest (45%) incidence of *S. assamensis* larvae in *M. pattoni* was recorded in August and the lowest (16%) in December. No larvae could be found in January and February.

Odeyemi (2005) Petroleum ether extracts of leaves and kernels of the neem plant, *Azadirachta indica* A. Juss, mixed at varied dosages of 0, 50, 150, 250, 350 and 500 mg/ml with decorticated groundnut seeds (50 g), were tested for their potential to control the Khapra beetle, *Trogoderma granarium* Everts. The kernel extract was more potent than the leaf extract. The number of emerged adults decreased with increasing extract concentrations for both leaves and kernels. The number of emerged adults in the control (72.2 ± 4.83) was significantly higher ($P < 0.05$) than all neem extract variants. The mortality of adults and larvae increased with increasing extract concentrations from 36.42 ± 4.62 % in the control to 60 ± 2.24 % at 500 mg/ml for leaf extract and to 55.08 ± 3.62 % at 500 mg/ml for kernel extract. Seed damageexpressed as the number of holed seeds and weight loss of infested groundnut was significantly reduced ($P < 0.05$) in each treatment as dosages increased. There was no significant reduction in the viability of

groundnut seeds treated with neem extracts when compared with the control. Regression analysisshowed positive correlations between dosages, adult and larval mortality and seed germination in each treatment. A negative relationship was observed between the dosages and emergence of offspring, seeddamage and weight loss for each treatment.

Chaube *et al.* (2006) The NLE induced morphologic apoptotic changes such as shrinkage, membrane leakage, and cytoplasmic fragmentation prior to degeneration of oocytes. The NLE-treated oocytes that had morphologic apoptotic features showed overexpression of bax protein and DNA fragmentation as evidenced by terminal deoxynucleotidyl transferase nick-end labeling-positive staining and DNA ladder pattern.

Mostofa *et al.* (2007) He examined *Catharanthus roseus* (Nyantara), *Azadirachta indica* (Neem), *Allium sativum* (Garlic) are medicinal plants, used in Ayurveda for treating various diseases, one of which is diabetes mellitus. In the present study of 12 months period from January to December 2007, aqueous extract of this plants were prepared and blood glucose lowering effect and improvement of body weight gain in Streptozotocin (50 mg/kg bwt i.p.) induced diabetic rats were measured and compared with that of a patent drug glimepride in the Department of Pharmacology, Bangladesh Agricultural University, Mymensingh. Rats were administered *Catharanthus roseus*, *Azadirachta indica*, *Allium sativum* extracts at the dose rate of 1g/kg, 500 mg/kg and 1g/kg bwt orally for 14 days, respectively. Blood glucose level and body weight was measured by Glucotrend kit and Electronic balance and that compared with a patent drug Glimepride at a dose rate of 100 mg/kg bwt. The data were compared statistically by using student's unpaired t-test. The herbal preparations of these plants significantly increased body weight gain and decreased blood glucose as compared with the patent drug. The present study clearly indicated the significant antidiabetic activity of *Catharanthus Roseus*, *Azadirachta indica* and *Allium sativum* and supports the traditional usage of the herbal preparations by Ayurvedic physicians for the therapy of diabetics.

Rahman *et al.* (2009) the experiment was carried out for 28 days from August to September 2007 to investigate the efficacy of Ivermectin and Neem plants against ectoparasites in calves. Effect of Ivermectin and Neem plants on certain blood parameters hemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TEC) and erythrocyte sedimentation rate (ESR) were studied in calves. A total of 15 calves were examined for the presence of ticks by physical examination and were divided into three equal groups as, Group A (infected control group), Group B (treated with Neem) and Group C (treated with Ivermectin). The therapeutic efficacy of Ivermectin was 100% against ectoparasites in calves on day 7, 14, 21 and 28 after the treatment of Ivermectin. Ivermectin and Neem showed significant effectiveness at 7^{th}, 14^{th}, 21^{st} and 28^{th} day of post treatment. The effects on TEC, Hb level was increased significantly at 7^{th}, 14^{th} and 28^{th} day of treatment and PCV level was increased significantly on 28th day of treatment. On the other hand indigenous medicinal plant Neem leaves shown the efficacy of 68% at day 28 and body weight of Ivermectin and Neem treated calves also increased. All the calves after Ivermectin injection and Neem spray remained healthy, no adverse effect and calves appetite increased, growth and coat color improved rapidly.

Rai *et al.* (2010) examined Stephanofilariasis, one of the most problematic parasitic diseases caused by Stephanofilaria assamensis, is endemic to Andaman and Nicobar Islands and many other states of India as well as neighbouring countries. The present study was conducted to find out the prevalence of the disease in the endemic areas of the islands, its economic impact and suitable measures for its eradication. The prevalence of the disease was varied from 10.2 to 70.5%. The incidence rate was more in older animals. The control and eradication of stephanofilariasis in bovines could be successfully achieved especially by targeting the life cycle of the biological vector (Musca conducens), the intermediate host. **N.S. Randhawa *et al.* (1996)**

2.1 Toxonomic position of Neem:

Kingdom: **Plantae**

Division: **Magnoliophyta**

Order: **Sapindales**

Family: **Meliaceae**

Genus: ***Azadirachta***

Species: ***A. indica***

Binomial name *Azadirachta indica*

2.2 Introduction of Neem:

Azadirachta indica (Neem) is a tree in the mahogany belonging to the family Meliaceae. It is one of two species in the genus *Azadirachta*, and is native to India, Pakistan, and Bangladesh growing in tropical and semi-tropical regions. Neem is a fast-growing tree that can reach a height of 15–20 metres (49–66 ft), rarely to 35–40 metres (115–130 ft). It is evergreen, but in severe drought it may shed most or nearly all of its leaves. The branches are wide spread. **Zillur *et al* (1993)**

2.3 Neem Dosing: There are inadequate clinical trials to support specific therapeutic dose of Neem. **Zillur *et al* (1993)**

2.4 Chemistry

The seed kernels of neem yield about 10% of a fixed oil, comprised primarily of glycerides. The yellow, bitter oil has a garlic-like odor and contains approximately 2% of bitter principles including azadirachtin, azadiradione, azadirone, gedunin, nimbidin, nimbin, ninmbolide, nimbinin, nimbidol, margolene, mahmoodin, salanin, meldenin, vepinin, and other related limonoid triterpenes. Azadirachtin is the most active

insecticidal component of neem, with a yield of about 5 g from 2 kg of seeds. All parts of the tree yield beta-sitosterol. The leaves also contain quercetin, gallic acid, catechin, carotenes, and ascorbic acid. 2 Low concentrations of aflatoxin have been reported. **Subapriya R, Nagini S. Medicinal properties of neem leaves: a review. Curr Med Chem Anticancer Agents. (2005)**

2.5 Chemical compounds

Salimuzzaman Siddiqui was the first scientist to bring the anthelmintic, antifungal, antibacterial, and antiviral constituents of the Neem tree to the attention of natural products chemists. In 1942, he extracted three bitter compounds from neem oil, which he named as nimbin, nimbinin, and nimbidin respectively. The process involved extracting the water insoluble components with ether, petrol ether, ethyl acetate and dilute alcohol. The provisional naming was nimbin (sulphur-free crystalline product with melting point at 205 °C, empirical composition $C_7H_{10}O_2$), nimbinin (with similar principle, melting at 192 °C), and nimbidin (cream-coloured containing amorphous sulphur, melting at 90–100 °C). Siddiqui identified nimbidin as the main active antibacterial ingredient, and the highest yielding bitter component in the neem oil. These compounds are stable and found in substantial quantities in the Neem. They also serve as natural insecticides. **Razzaghi-Abyaneh *et al* (2005)**

2.6 Mode of action:-

In insects, it exhibits various behavioural responses such as Antifeedant. Feeding Deterrent, Oviposition Deterrent etc., and physiological responses such as insect growth regulator, molting inhibitor, reproduction inhibitor, antifertility etc. **Sinniah D, Baskaran G. Margosa (1981)**

2.7 Medicinal use

Neem products are believed to be anthelmintic, antifungal, antidiabetic, antibacterial, antiviral, contraceptive and sedative. It is considered a major

component in Ayurvedic and Unani medicine and is particularly prescribed for skin disease. Ozoneem Aza is our brand for Azadirachtin Technical. It is yellowish powder that contains 15% to 25% Azadirachtin. **Sinniah D, Baskaran G. Margosa (1981)**

2.8 Bioactivity

It acts as a broad spectrum biopesticide controlling a large number of insect including caterpillars, beetles, whiteflies, leafhoppers, aphids, mites, thrips, borer, mealy bug, leaf folders and many more. **Sinniah D, Baskaran G. Margosa (1981).**

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 Study area

The experiments were conducted for a period of 5 months from July/2012 to November/ 2012 at Veterinary Hospital, Hajee Mohammad Danesh Science and Technology University, Dinajpur and also villages of Gornurpur, Khalpara, Boalpatma, Itua, Maklafata of Kaharole upazila under Dinajpur district.

3.2 Selection of cattle

A total of 86 cattle of both sexes of different ages were examined during the period of July to November/ 2012 to study the prevalence of humpsore. The site of lesion as well as age, sex and breed were recorded in all the animals showing evidence of stephanofilariasis. The lesions were confirmed by clinical findings. Out of 86 cattle, 36 were examined in the Veterinary Hospital at Hajee Mohammad Danesh Science and Technology University, Dinajpur. The rest 50 were examined in the nearby villages-Gornorpur, Khalpara, Boalpatma, Itua, Maklafata of Kaharole upazila under Dinajpur district.

3.3 Experimental layout

15 clinical cases of stephanofilarial dermatitis having typical humpsore lesion on hump and neck region were selected for study purpose. The lesions of humpsore were 3.5-10.5 cm in diameter.It was measured with the help of slide calipers. Smaller lesions were considered less than 5 cm and larger lesions were considered greater than 5 cm. All the cattle were mostly cross-breed progeny of Holstein Friesian, Shahiwal and some local breed aging from 4 to 12 years. The draft purpose cattle were affected more. These 15 cattle were divided into 3 groups each consisting, of 05 cattle. One group (group C) was kept as untreated control group and rest two were treated with Neguvon and Neem leaves extract respectively. All the cattle were maintained with balanced feed and water *ad libitum*.

Experimental layout

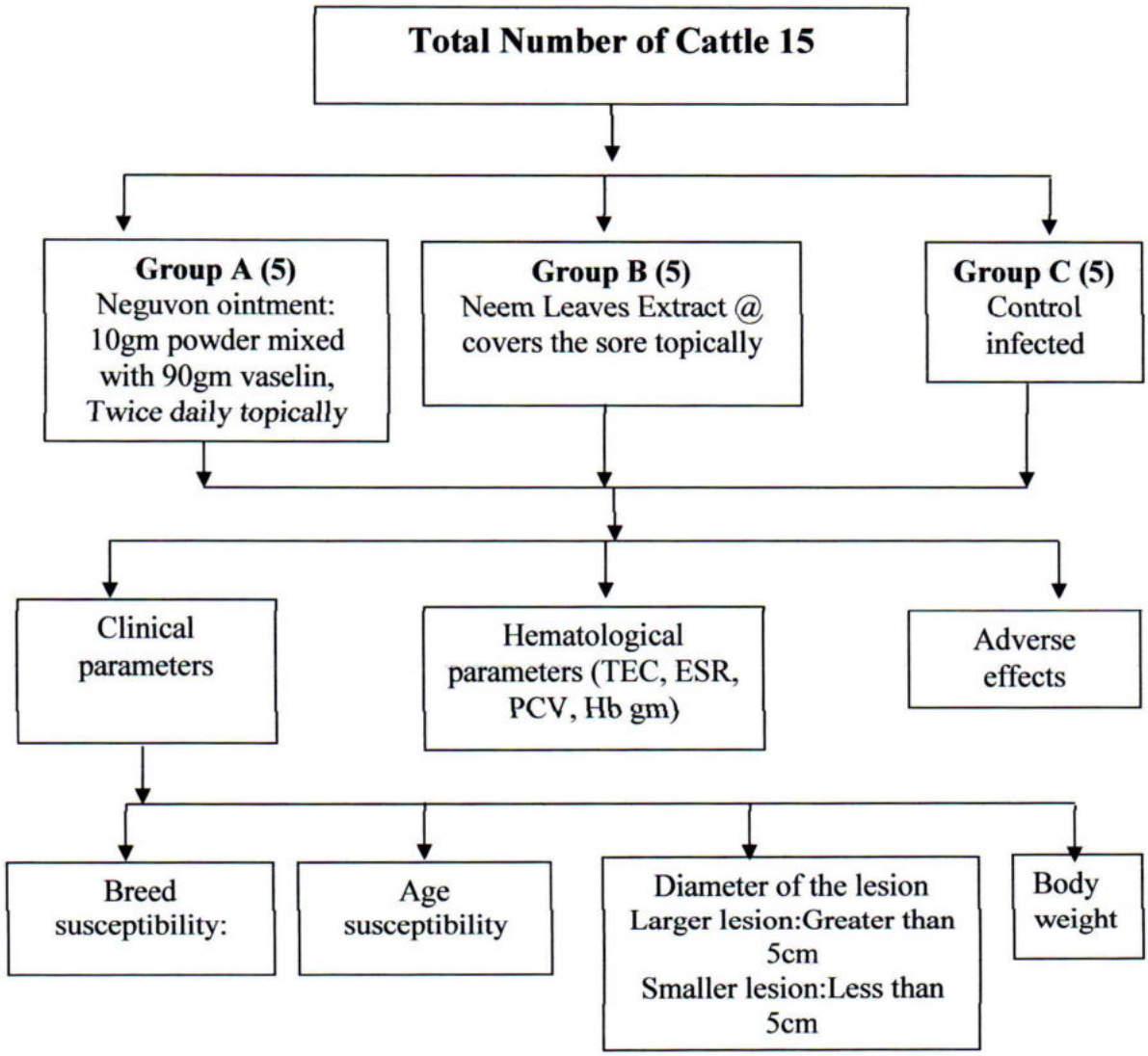


Fig.1. Experimental layout

3.4 Materials.

3.4.1 Neguvon (R)

Neguvon (R) (Trichlorphon, Bremer, Pharma, Germany), ointment and neem leaves were collected from Hajee Mohammad Danesh Science and Technology University campus and also from nearby villages.

Photograph I : Neguvon (R) (Trichlorphon, Bremer, Pharma, Germany), ointment

3.4.2 Preparation of Neem leaves extrct

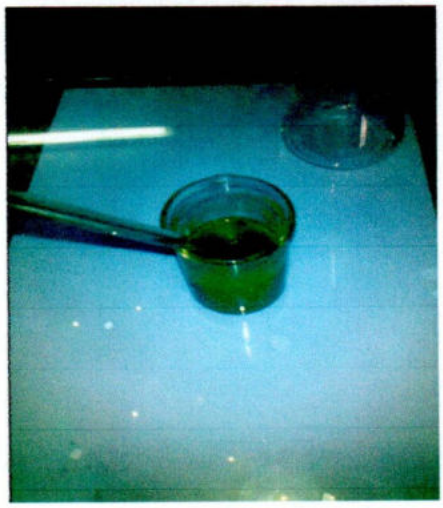
Neem leaves (*Azadirachta indica*) were collected from the campus of Hajee Mohammad Danesh Science and Technology University, Dinajpur.

To obtain 15% aqueous extract, 15gm of Neem leaves were weighted from electric balance then thoroughly washed in tap water. The leaves were cut into small pieces with the help of knife, there after the fleshy parts were mashed with the help of mortar and pastels. The extract was made up to 100ml by adding distilled water and filtered it through the filter paper with the help of Beaker and Funnel. It was kept overnight at 40 degree centigrade and the supernatant was collected.

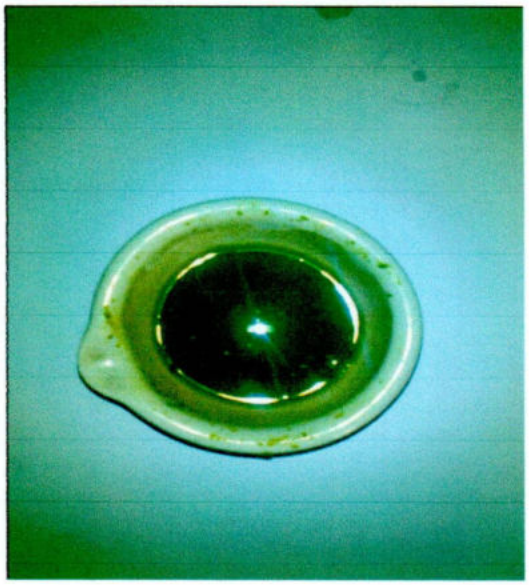
Photograph II: Neem Leaves

Photograph III: Weighted by electric balance

Photograph IV:Maceration



Photograph V: Added water to neem



Photograph VI: Neem Leaves extract

Photograph VII: Filtration

Photograph VIII: Collect Neem leaves extract

3.5 Application of drugs

Neguvon (R) ointment was applied topically twice daily to the cattle for 28 days of the cattle of Group-A. 10 gm Neguvon powder was mixed with 90 gm vaseline. And 15% neem extract were used in affected area topically. The fate of the lesions was inspected in two groups every alternative day for one month from the first application of the drugs. Efficacy of Neguvon and Extract of Neem leaves was assessed by observing clinical healing of lesions, and period of healing of the lesions.

3.6 Effect on body weight

The body weight of all treated and control groups of affected cattle were recorded before application of Neguvon (R) and Neem leaves extract and on day 30 after application of drugs.

3.7 Effect on blood

Blood samples were collected from Jugular vein of the cattles of treated and control group in vials containing anticoagulant (Sodium Citrate 3.8%) at 1st, 7th, 14th and 28th day of treatment period to determine the effects of Neguvon (R) and neem on the following hematological parameters such as Total Erythrocyte Count (TEC), Hemoglobin (Hb) Content, Packed Cell Volume (PCV) and Erythrocyte Sedimentation Rate (ESR).

3.7.1 Total Erythrocyte Count (TEC)

Thomas red blood cell pipette was used for Total erythrocyte count (TEC). The pipette was filled up to 0.5 marks with blood and diluting fluid (Hayem's solution) was then drawn till it reached the 101 mark. The contents were thoroughly mixed for 2 minutes. The dilution of the contents was 1:200. The counting chamber and cover slip were cleaned and dried properly. The cover slip was placed on the chamber and cover slip were cleaned and dried properly. The

cover slip was placed on the chamber in proper position. The content of the pipette was again shaken and 2 or 3 drops of the fluid were expelled. The chamber was then filled with the contents so that the fluid was flown under the cover slip by capillary action. The ruled area at the chamber was filled completely, taking care that excess did not run into the troughs and no air bubble appeared under the cover slip. The counting chamber was then placed under microscope and examined first with low power objectives (10x) to ensure that there was an even distribution of the cells. The cells were then counted with the aid of high power objectives (100x). The central squares of the counting chamber were counted in the far corner and one central square of the chamber. The counting and calculation of red blood cells were performed as per methods indicated by Coffin (1955). The number of RBC was calculated as follows: Number of RBC = No. of cell counted × 10,000 and the result was expressed in million/cu.mm. **(Coffin (1955).**

3.7.2 Determination of Hemoglobin (gm %)

The hemoglobin estimation was performed by the acid Haematin method with the Hellige Hemometer (Coffin, 1955). Hydrochloric acid (N/10) solution was taken in the special graduated tube upto its 20 mark. The special Sahli pipette was filled with well mixed oxalated blood upto 20 mark and blood on the side of the pipette was wiped out by cotton. The content of the Sahli pipette was expelled into the special graduated diluting tube and thoroughly mixed. The tube was then allowed to stand for 10 minutes for development of acid haematin. Distilled water was then added drop by drop and each time mixed with the help of stirring rod until the colour of the solution matched with the standard colour of the comparator. The result was read as per method described by **(Coffin, 1955).**

3.7.3 Packed cell volume (PCV)

The PCV was determined as per method described by (Coffin, 1955). The wintrobe haematocrit tube was filled up with well-mixed blood by special loading pipette upto 10 marks. Then the tube was centrifuged at 3,000 rpm for half an hour and reading was taken.

3.7.4 Determination of Erythrocyte Sedimentation Rate (ESR)

The collected blood sample was filled with the special loading pipette slightly above the '0' mm mark or '10' mark. The tip of the pipette was inserted to the bottom of the haematocrit tube and the blood was expelled by the pressure on the rubber bulb, withdrawn the pipette as the tube filled. Each tube was then placed in the special rack being certain that they were vertical. The tube was allowed to remain in the support without disturbance for an hour. The ESR value was determined as per methods describe by (Coffin, 1955).

CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

A research work was conducted -

1. To study the comparative efficacy of Neguvon and Neem leaves extract against humpsore in cattle.
2. To determine the effect of Neguvon and Extract of Neem leaves on some clinical parameters (severity of infestation, body weight, hair coat, and adverse effect) in cattle.
3. To determine the effect of Neguvon and Extract of Neem leaves on some hematological parameters (TEC, ESR, PCV, Hb) in cattle.

4.1 The prevalence of stephanofilariasis (Humpsore) in cattle of Veterinary Hospital at Hajee Mohammad Danesh Science And Technology University and nearby five villages of Dinajpur district.

Out of 86 cattle of Veterinary Hospital at Hajee Mohammad Danesh Science and Technology University and near by four villages - 15 were found affected with stephanofilariasis. Out of 15 positive cases the site of the disease was distributed over the neck region (29.12%) hump region (7.5%) and other (2.48%). The prevalence was recorded (39.1.2%). high case of male animals the lesions were mainly in the neck region. It was also observed that draft purpose cross-breed cattle were more susceptible to the disease than local breed.

4.2 To study the comparative efficacy of Neguvon(R) and Neem leaves extract against humpsore in cattle.

4.2.1 Neguvon

Stephanofilariasis of group A cattle (photo IX & X) treated with Neguvon (R) topically twice daily showed gradual improvement of the lesions within 7th post treatment days and marked improvement of the lesions within 21st days of treatment which was assessed by the gradual reducing of irritation and size of the lesions and formation of crust to the sore. At the end of the 24th days of post treatment days the lesions were completely healed up with the formation of scar tissue and then new hair started to grow over the entire affected area (Table I, Figure IX and X).

Photograph IX: Humpsore lesion before treatment with Neguvon powder.

Photograph X: Humpsore lesion after treatment with Neguvon powder.

4.2.1.1 Table 1. Effect of Neguvon ointment and Neem leaves extract aginst humpsore in cattle

Experimental Days	Diameter of sore (cm) in Group A after application of Neguvon (mean±SE)	% of Reduction	Diameter of sore (cm) in Group B after application of Neem leaves extract (mean±SE)	% of Reduction	Diameter of sore (cm) in Group C (Control group) (mean±SE)	% of Reduction
0	5.8±.13	0	6.24±.16	0	6.4±.31	0
5th	3.5±.17	39	4.58±.12	27	6.4±.34	0
9th	2.2±.11	62	3.03±.11	52	6.5±.35	0
13th	1.1±.12	81	2.04±.21	67	6.6±.36	0
17th	0	90	1.21±.13	82	6.7±.37	0
21st	0	97	1.08±.04	90	6.8±.39	0
25th	0	100	0.97±.06	96	6.9±.40	0
28th	0	0	0	100	7.0±.41	0
30th	0	0	0	0	7.1±.42	0

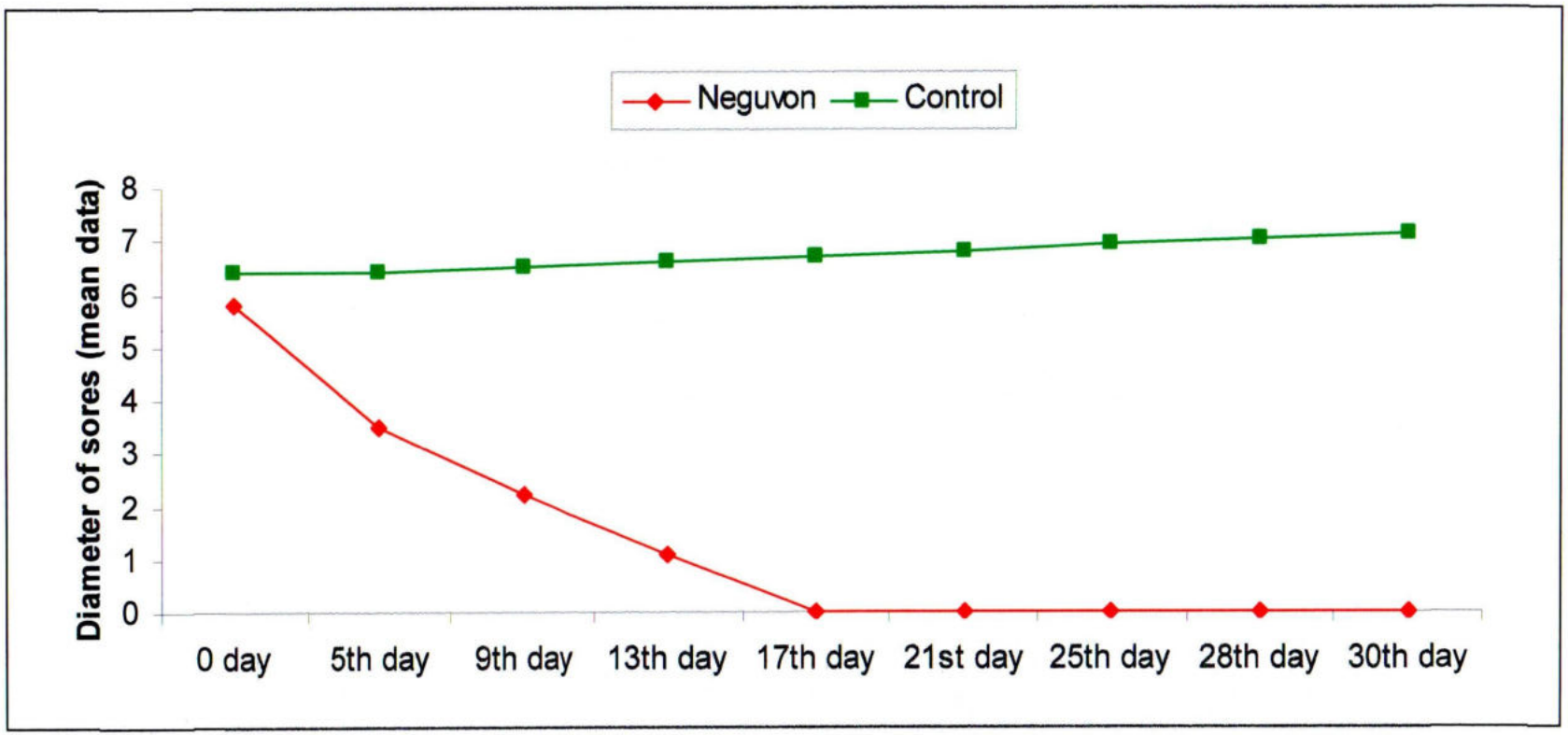
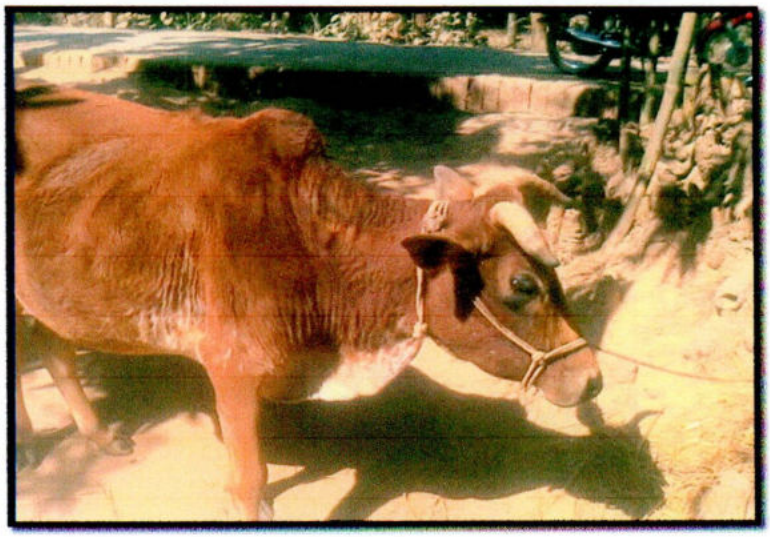


Fig. 02. Effect of Neguvon ointment with control group aginst humpsore in cattle

4.2.2 Neem leaves extract

Stephanofilariasis of group B cattle (photo XI & XII) treated with Neem leaves were used to covers the sore area topically around the sore showed slight improvement after the application which was assessed by reduction of itching, irritation and progressive drying. After application of 2-7th day there was significant improvement with reduction of the size of the lesions. After 14th days post treatment lesions were completely healed up by formation of smooth black scar tissue within 29th days. It was also observed that smaller lesions improved more rapidly than the larger lesions. (Table 1, Figure XI, XII).

Photograph IX: Humpsore lesion before treatment with Neem leaves extract.



Photograph XII: Humpsore lesion after treatment with Neem leaves extract

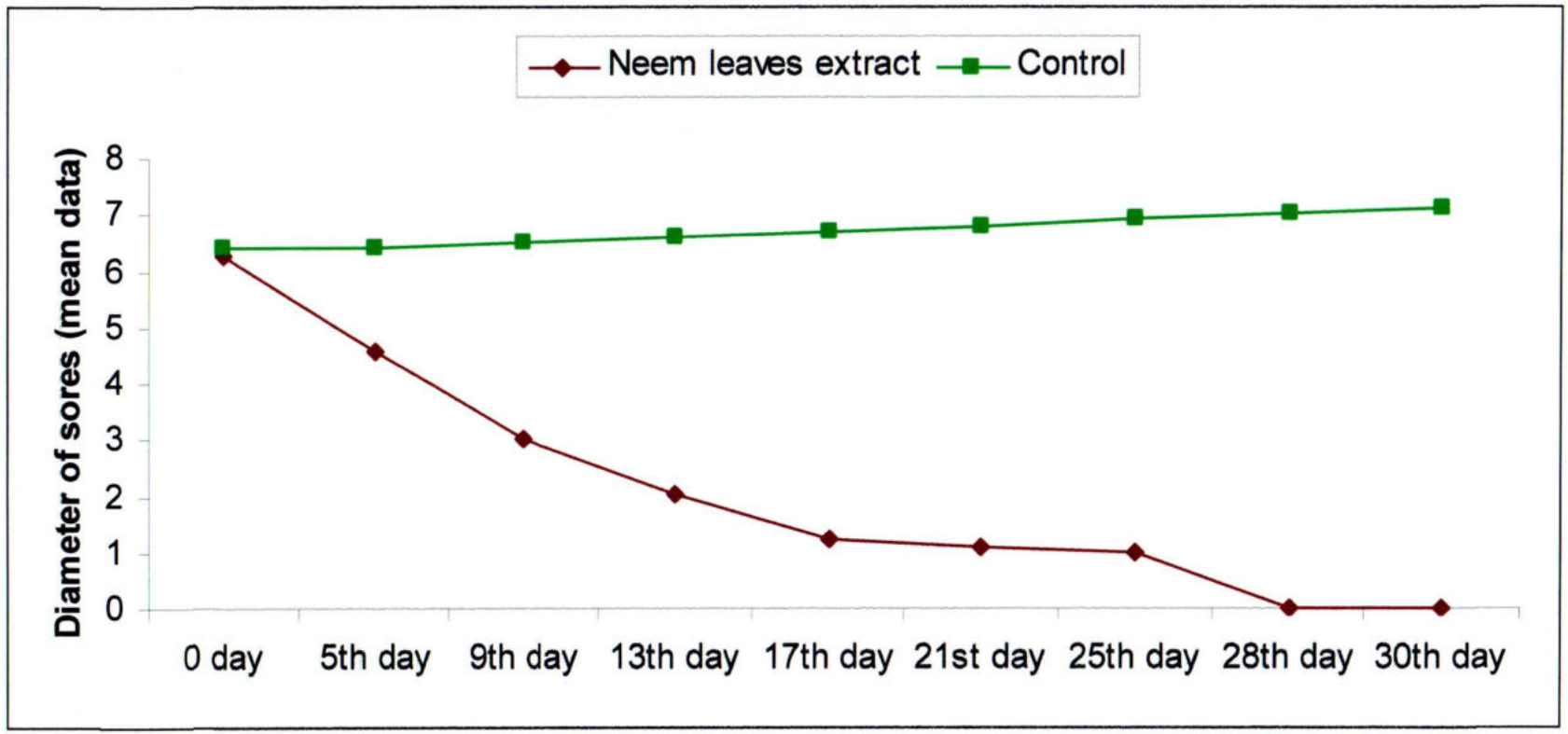


Fig. 03. Effect of Neem leaves extract with control group aginst humpsore in cattle

4.2.2.1Effect of Neguvon ointment and Neem leaves extract with control group aginst humpsore in cattle

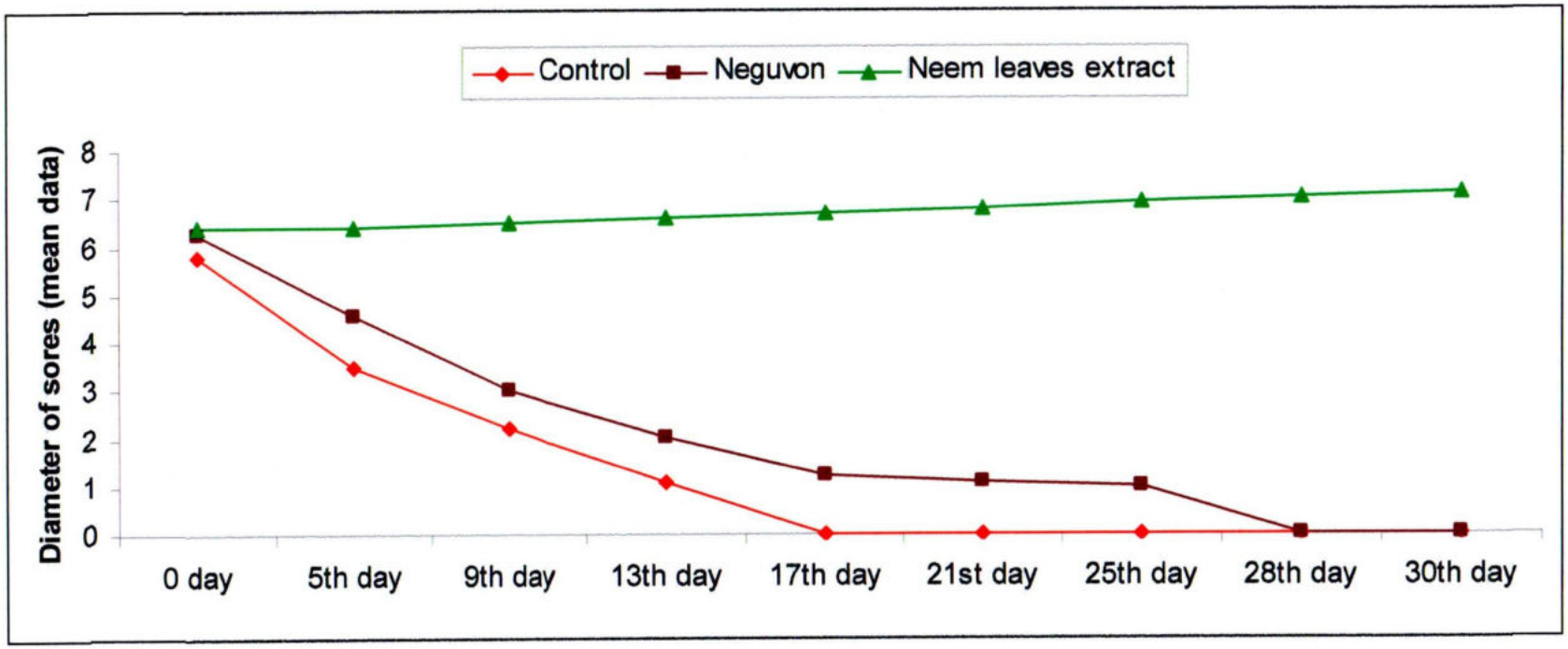


Fig. 04. Effect of Neguvon ointment and Neem leaves extract with control group aginst humpsore in cattle

4.2.3 Comparative efficacy of Neguvon and Neem leaves extract

It was also observed that treatment with Neem leaves extract required 29 days to complete recovery of the sore. On the other hand topical application of Neguvon showed 100% effectiveness at the end of the 24 days. The larger lesions required longer time to heal up then that of smaller lesion when treated with Neem leaves extract but the recovery time was shorter when treated with Neguvon

No side effect or Adverse reaction was produced by any of the formulation (Neem leaves extract and Neguvon) used in this experiment. The results of therapeutic trials are represented in Table I. The lesions (humpsore) of animals of the untreated control group (group C did not show any improvement during the period of the experiment).

4.3 Effect of Neguvon and Neem leaves extract on body weight and Hair coat

The body weight of treated cattle (Group A & B) was increased to the extent of 3.9% and 2.5% on 30^{th} day, following Neguvon and Neem leaves extract application respectively. On the other hand, the body wt. of control group decreased gradually (Table II).

4.3.1 Clinical examination of cattle

Clinical examination of all group of cattle except control showed normal rectal temperature, pulse and respiration after treatment with Neguvon and Neem leaves extract.

4.3.3 Effect of Neguvon powder on body weight in cattle

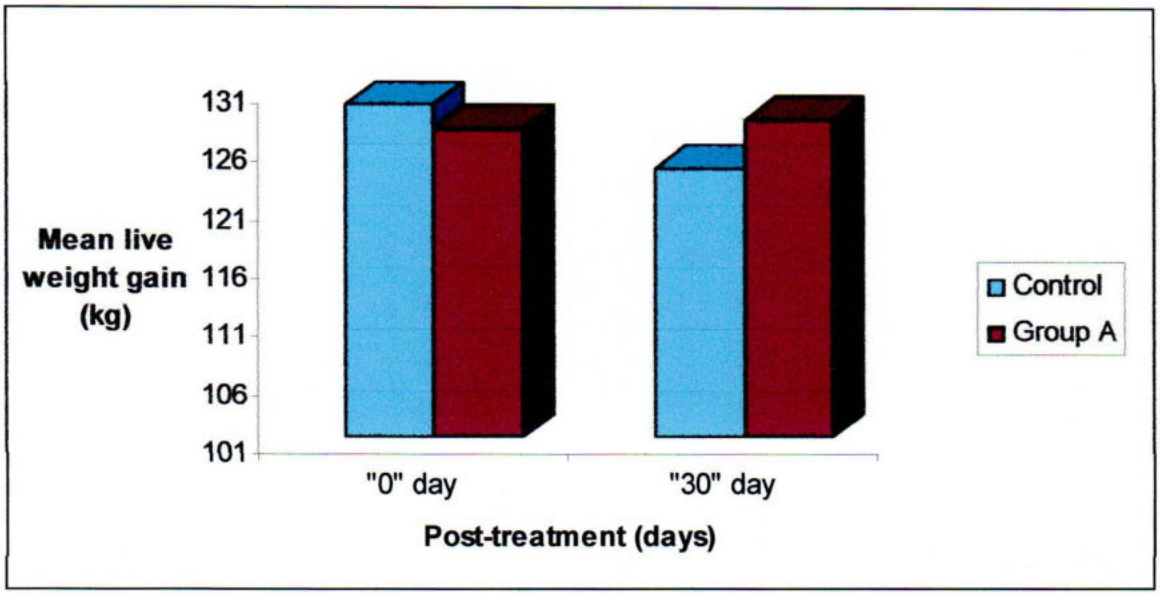


Fig. 05. Effect of Neguvon powder on body weight in cattle

4.3.4 Effect of Neem leaves extract on body weight in cattle

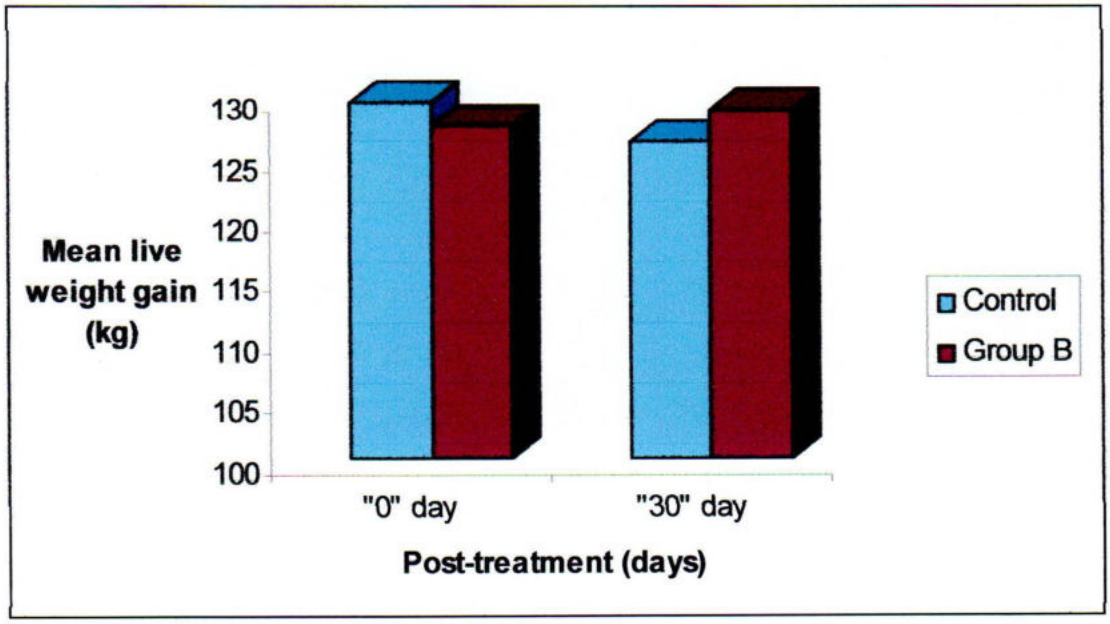


Fig.06. Effect of Neem leaves extract on body weight in cattle

4.3.5 Effect of Neguvon and Neem leaves extract compeering with control group on body weight in cattle

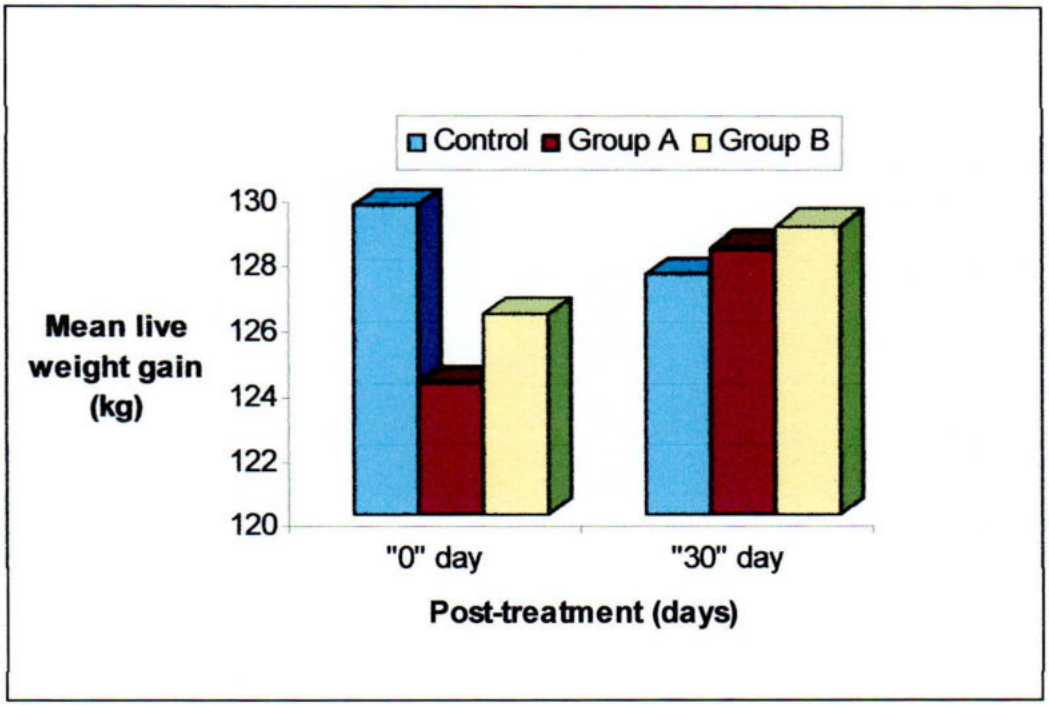


Fig.07. Effect of Neguvon and Neem leaves extract compeering with control group on body weight in cattle

4.3.6 Effect of Neguvon and Neem leaves extract on hair coat

To evaluate the anthelmintic activity and certain blood parameters of Neem leaves extract and Neguvon® in comparison with the control group. In Group B, Neem at 1gm or 15% spray was found to be 68% effective against humpsore. Ticks were decreased within the selected area on 7^{th}, 14^{th}, 21^{st} and 28^{th} day after the treatment on the other hand in control group C, the number of ticks increased gradually on 7^{th}, 14^{th}, 21^{st} and 28^{th} day of treatment..

The hair coat of the infected cattle (group A and B) was rough with discolored wool on 1st day. In group A and B, after treatment with Neem leaves extract and Neguvon (R), the hair coat started to become smooth and shiny gradually and on 28th day of treatment the hair coat of the treated cattle were almost normal. The hair coat of the infected control group became more rough and discolored. The mean initial body weight of group A and B treated with Neem leaves extract 15% solution or to covers the sore area and Neguvon (R) (200µg/kg) or 10gm mixed with 90 gm vaseline were 126.2 kg and 124.10 kg on day 0 and on the 28th day of post treatment the mean values of body weight were 128.90kg and 128.25 kg, respectively. So body weight of cattle increased significantly in treated groups on 28th day of treatment. In control group (C), body weight of cattle was decreased upto 6.63% on 28th day of treatment. On the other hand, the body weight was increased in groups (A and B) to the extent of 2.50%-3.90%. So body weight of cattle increased significantly in treated groups on 28th day of treatment (Table 2).

4.4 To determine the effect of Neguvon and Extract of Neem leaves on some hematological parameters (TEC, ESR, PCV, Hb) in cattle.

4.4.1 Table 3. Effects of Neguvon and neem leaves on TEC (million/cu. Mm) in cattle

Group	Treatment	Pre-unit treatment	Unit Post-treatment			
		0 day	7th day	14th day	21st day	28th day
A	Neguvon	6.8±0.084	8.32 ±0.128** (18.27)	9.046 ±0.072** (8.03)	10.364 ±0.162* (12.72)	11.42 ±0.169* (9.25)
B	Neem	6.47±0.156	7.476 ±0.074 (13.46)	8.91 ±0.213** (16.09)	10.068 ±0.078 (11.50)	10.62 ±0.186** (5.20)
C	Control	7.16±0.108	6.72 ±0.116 (6.55)	6.46 ±0.150 (4.02)	6.38 ±0.13 (1.25)	6.54 ±0.154 (2.45)

Values given above are mean±SE of 05 cattle * significantly increased (P<0.05), ** significantly increased (P<0.01)

In control group (C), hemoglobin content was decreased upto 4.52% on 28th day of treatment. On the other hand, the hemoglobin contents were increased in treated groups A and B to the extent of 1.10%-3.02%, respectively. So hemoglobin content was increased significantly in treated groups on 28th day of treatment (Table 4). In control group (C), PCV (%) values were decreased upto 14.07% on 28th day of treatment. On the other hand, the PCV (%) values were increased in all treated groups (A and B to the extent of 1.11%-1.45% (Table 5). In control group (C), ESR (mm/1st hr) values were increased up to 5.19 on 28th day of treatment. On the other hand, the ESR (mm/1st hr) values were decreased in all treated groups of A and B significantly ($P<0.001$) (Table 6).

4.4.2 Table 4. Effects of Neguvon and neem leaves on Hemoglobin Hb content (gm %) in cattle

Group	Treatment	Pre-unit treatment	Post-treatment			
		0 day	7th day	14th day	21st day	28th day
A	Neguvon	6.46±0.144	7.32 ±0.097** (11.75)	7.56 ±0.150** (3.17)	8.34 ± 0.129* (9.35)	8.6 ± 0.195* (3.02)
B	Neem	7.14±0.117	7.38 ±0.116** (3.25)	7.66 ±0.108** (3.66)	8.60 ± 0.145** (10.97)	8.7** ± 0.114 (1.10)
C	Control	6.58±0.420	7.082 ±0.197 (6.98)	6.62 ±0.180 (6.88)	6.88 ± 0.193 (3.80)	6.59 ±0.168 (4.52)

Values given above are mean±SE of 05 cattle, * significantly increased (P<0.05), ** Significantly increased (P<0.01).

4.4.3 Table 5. Effects of Neguvon and neem leaves on Packed cell volume (PCV) (%) values in cattle

Group	Treatment	Pre-treatment	Post-treatment			
		1st day	7th day	14th day	21st day	28th day
A	Neguvon	29.75±0.307	29.58 ±0.229** (0.57)	29.49 ±0.159** (0.31)	30.69 ±0.433** (3.93)	30.36 ±0.469** (1.11)
B	Neem	28.41±0.349	28.50 ±0.318** (0.34)	28.892 ± 0.173** (1.33)	29.82 ±0.887 (3.11)	29.39 ±0.381 (1.45)
C	Control	28.99±0.909	29.63 ±0.259 (2.17)	29.2 ±0.251 (1.49)	26.44 ±0.267 (10.44)	23.18 ±0.295 (14.07)

Values given above are mean±SE of 5 cattle, * significantly increased (P<0.05), ** Significantly increased (P<0.01)

4.4.4 Table 6. Effects of Neguvon and neem leaves on Erythrocyte sedimentation rate (ESR mm/1st hr)

Group	Treatment	Pre-treatment	Post-treatment			
		1st day	7th day	14th day	21st day	28th day
A	Neguvon	0.146±0.010	0.11 ±0.009 (30.91)	0.0916±0.021** (20.09)	0.083±0.013* (10.36)	0.030±0.007**(76.67)
B	Neem	0.118±0.011	0.0854 ±0.014 (41.69)	0.060±0.004** (42.33)	0.050±0.007 (20.00)	0.036±0.011**(38.89)
C	Control	0.944±0.019	1.034 ±0.012 (9.28)	1.404±0.017 (26.35)	1.828±0.026 (23.19)	1.928±0.033 (5.19)

Values given above are mean±SE of 05 cattle, * Significantly increased (P<0.05), ** Significantly increased (P<0.01)

CHAPTER V

DISCUSSION

CHAPTER V

DISCUSSION

The present research work was performed at the Veterinary Hospital at Hajee Mohammad Danesh Science and Technology University, Dinajpur and also examined in the nearby villages Gornurpur, Khalpara, Boalpatma, Itua, Maklafata

Prevalence of stephanofilariasis in Veterinary Teaching Hospital of Hajee Mohammad Danesh Science and Technology University And nearby five villages-

Out of 86 cattle of Veterinary Teaching Hospital at of Hajee Mohammad Danesh Science and Technology University and nearby five villages, 30were found affected with stephanofilariasis. Out of 15 positive cases the site of the disease was distributed over the neck region (29.12%), hump region (7.5%) and other (2.48%). The prevalence was recorded as 39.12%. In case of male animals the lesions were mainly in the neck region. It was also observed that draft purposes cross breed cattle were more susceptible to the disease than local breed. The present findings were similarly as reported by Alam (1995).

5.1 Effect of Neguvon and Neem leaves extract agains and humpsore in cattle:

5.1.1 Neguvon

The topically applied Neguvon powder was 100% effective against stephanofilariasis in cattle without showing any side effect. The complete recovery was observed within 28th day of 1st day topical use. The present findings have close correlation with the findings of Baki and Dewan (1975). They topically applied Neguvon ointment (Neguvon–Sulfanilamide+Glycerine) and recommended it as a most effective drug. The findings of the present study were also correlated with Dewan and Baki (1976). They stated that Neguvon

sulfanilamide ointment (20%) Neguvon, 5% sulfianilanilde and 75% Vaseline) was very effective (100%) against Humpsore. In the present research study, neguvon was found less effective.

5.1.2 Neem leaves extract

Topical application of Neem leaves extract were used to covers the sore area topically .It was found 85% effective without any side effect. Animals with both larger and smaller lesions were cured within 29th days post treatment. After the 1st day treatment, the percentage of healing of the sore was less. In subsequent treatment on the 1st to 14th day the healing was remarkable with scar tissue formation and growth of new hair around the scar tissue. This may be due to the antiparasitic action of the Neem leaves extract.

5.2 Effect on body weight and hair coat

5.2.1 Neguvon

In the present study animal treated with Neem leaves extract increased 4.5% of live weight. Similarly Islam (1992) reported significant increase of body weight in cows and bull, buffaloes, treated with application of Neguvon also increased the body wt. up to 2.70%.

Kennedy noted (1990) noted that Neem leaves extract at the dose rate of 1gm/kg (15%) topical action showed weight gain.

In this research work, Neguvon used topically found to be more effective than Neem leaves extract against humpsore of cattle.

5.2.2 Neem leaves extract

Topical application of Neem leaves extract were used to covers the sore area. It was found 85% effective without any side effect. Animals with both larger and smaller lesions were cured within 29th days post treatment. After the 1st day

SUMMARY AND CONCLUSIONS

REFERENCES

REFERENCES

Agrawal M.C. (1977). Stephanofilaria saheeri . Singh, 1958. Concentration in ear sore lesions. *Oressa Veterinary Journal.***11**:122.

Ahmed Z. (1961) Comparative efficacy of neguvon and asuntal against Humpsore, *Bangladesh Journal of Veterinary Medicine* Vol **6**, No 1&2

Ahmed Z. (1961). The role of *Stephanofilaria zaheeri* in causing ear sore of buffaloes. *India Journal.* **38**: 257-262.

Alam M.A. (1995). Therapeutic efficacybbb of Fenitrothion and Neem seeds against Stephanofilariasis in cattle, M.S. Thesis, Department of Pharmacology, BAU, Mymensingh.

Anonymous T. (1985). Comparative efficacy of Ivermectin and asuntal against Humpsore, *Asian Livestock* .Vol **10**, No.05, PP.49

Baki M.A. (1995). Transmission and Pathology of Humpsore Ph.D. Thesis. Bangladesh Agriculture University, BAU, Mymensingh.

Baki M.A. and Dewan M.L. (1975). Evaluation of treatment of Stephanofilariasis (Humpsore) with Neguvon (Bayer) by clinico-pathological studies.*Bangladesh Veterinary Journal.***9**:1-6. *Bangladesh Journal of Veterinary Medicine* . Vol **5**, No 1&2

Brammer R.and Mel gibson (1996) Efficacy of ivermectin and Neem leaves against Stephanofilariasis.*Journal of veterinary Parasitology,* Vol:7,67-69

Buckley (1939) Efficacy of Ivermectin against humpsore,*World veterinary journal,***Vol:11 (2),**114-118

Chaube S.K., Prasad P.V., Khillare B., Shrivastav T.G. (2006). Extract of *Azadirachta indica* (neem) leaf induces apoptosis in rat oocytes cultured in vitro, Fertil Steril. **1:**1223-31.

Gopal Krishman (1948) Stephanofilariasis in buffaloes.*Veterinary journal* .Vol 7:232-278

Hansen (1964) Stephanofilarial lesions were observed on the ear, udder shoulder and medial canthus of eye of Jersy cows in Denmark, *World veterinary journal.*Volume **02:** 643-679

Hakim A. (2002) Efficacy of Neem leaves and Neguvon against Humpsore in Cattle.*Bangladesh journal of Veterinary Medicine*

Hasan (1964) Effect of Zinc chloride solution on humpsore in cattle, *Bangladesh Journal of Veterinary Medicine* Vol 7, No 1&2

Hannan and Mostofa(2001) Comparative efficacy of Neem leaves and Ivermectin against ectoparasite in calves.*Journal Bangladesh Agriculture University* Vol **7(1)**,No.73-78

Hdz and Adiwinata (1957) Effect of Antiniosmi against *stephanofilaria deodesi.*, *Journal of Veterinary Parasitology*, Volume: **29**, 92-122

Hiregauder and Chatupale (1965) Effect of neguvon and ivermectin against Stephanofilariasis, *Indian journal of animal sciences*. **80(6)**: 24-30

Hyldgara-Jansen and Dollar (1961) Effect ofneguvon Stephanofilariasis.*Journal of Ethnopharmacol.*Vol.;93(1):**33-56**

Johnson and Toleman (1988) The factors for the presence of stephanofilarial lesions in animal in Australia, *World veterinary journal.*Volume **10:**233-289

Mannan (1997) Effect of Ivermectin against Stephanofilariasis in Cattle, *Bangladesh Journal of Veterinary Medicine* Vol 7, No 3&2

Menon (1952) Stephanofilar[i]asis with solution and distilled oil of pinetar.,*World veterinary journal. Volume* **09:**133-189

Phukan S. Choudhury, Das M., Barkakoty M.R. (2005) Humpsore in cattle in Assam, *Journal of Veterinary Parasitology*, Vol: **19**, 19-22

Protap and Mishra(1987) In vitro antibacterial activity of Neem oil. *Indian J Med* Vol:**84**:314-316.

Rahim (1998) Effect of ivermectin on Stephanofilariasis in cattle,*Journal of Bangladesh Agriculture University*.Vol: **7(1):** 143–168.

Rahman M. M, Mostofa, Jahan M. S. and Kamal M. A. H. M. (2009). Comparative efficacy of Neem leaves and Ivermectin (Ivomec®) against ectoparasites in calves. *Journal of Bangladesh Agriculture University.* **Vol:7(1):** 73–78,

Rai, R. B., Neeraj, Srivasta,V.A., Jai, sunder, Kundu. A., Jeykumar. S. (2010) Stephanofilariasis in bovines: Prevalence, control and eradication in Andaman and Nicobar Islands, India. *Indian journal of animal sciences.* Vol:**80(6)**: 24-30

Razzaghi-Abyaneh M., Allameh A., Tiraihi T., Shams-Ghahfarokhi M. and Ghorbanian M. (2005). Morphological alterations in toxigenic Aspergillus parasiticus exposed to neem (*Azadirachta indica*) leaf and seed aqueous extracts. *Mycopathologia,* Vol:**159**: 565-570.

Round, (1964). Lesions of Stephanofilariasis in black rhinoceros *(Diceros bicornis), Journal of Veterinary Parasitology*, Vol**: 34**, 902-922

Roy and Misra (1991) Comparative efficacy of 1% ivermtin ointment, 0.5% ivermectin ointment and 7.5% levamisole ointment against Humpsore in cattle, , *Indian journal of animal sciences*.Vol: **90(6)**:129-150

Roy Choudhury and Chakrabarty, (1969). Effect of carbamazine citrate on otitis externa in bullock,*Indian journal of animal sciences*.Vol: **122(11)**:724-730