IN VIVO EFFECTS OF NEEM LEAVES EXTRACT AND IVERMECTIN AGAINST NATURAL TICK INFESTATION IN CALVES

A Thesis

By

MD. ZAHURUL ISLAM
Registration No. 1205121
Semester: July- December, 2014
Session: 2012-13

MASTER OF SCIENCE (M.S.)

IN

PHARMACOLOGY

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

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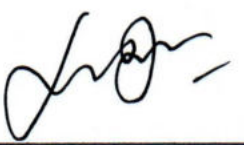
Approved as to style and contents by

(DR. Fahima Binthe Aziz)
Supervisor



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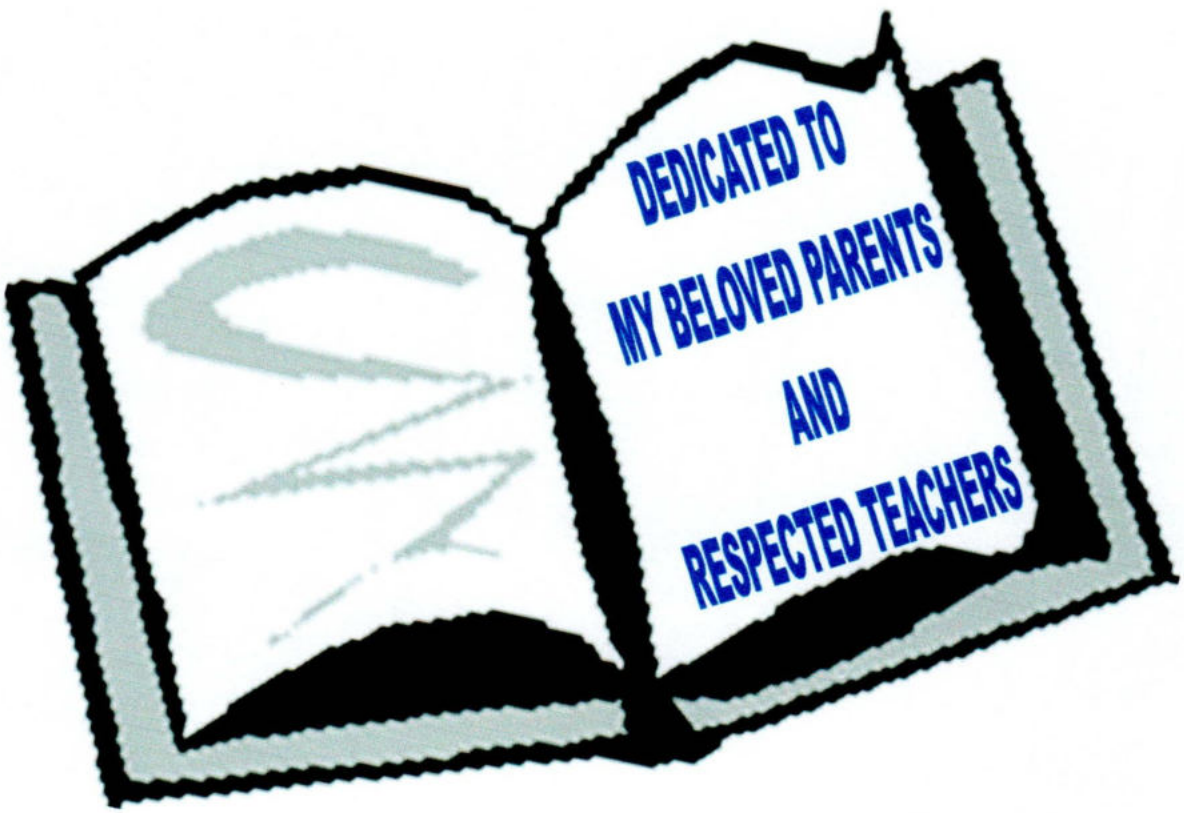
DEDICATED TO
MY BELOVED PARENTS
AND
RESPECTED TEACHERS

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ABSTRACT

Ticks are economically the most important ectoparasite of cattle and other domestic species in tropical and subtropical countries including Bangladesh. The present experiment was carried out to investigate the comparative efficacy of neem (*Azadirachta indica*) leaves extract (15% as spray) and ivermectin (1% as S/C injection) against tick infestation, their effects on some clinical and hematological parameters in calves. For this purpose 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). After spray of neem leaves extract and injection of ivermectin the treated and control groups were kept for 28 days and clinical and hematological parameters were investigated at 7 days intervals. On the basis of tick count, the efficacy of ivermectin was found 100% on day 7, 14, 21 and 28 after the treatment whereas neem leaves extract (spray) was 68.8% effective at day 28 against tick infestation in calves. The results showed that the body weight of calves increased after treatment in groups B and C respectively, but in control group body weight decreased. Compared to infected control groups, the feeding efficiency increased in all treated group. There was significant increase in hemoglobin concentration (Hb), packed cell volume (PCV%), and total erythrocyte count (TEC) and significant decrease in Erythrocyte sedimentation rate (ESR) in the neem and ivermectin treated group. All the calves after neem spray and ivermectin injection remained healthy and no adverse effect were observed. Appetite increased, and growth and coat color improved rapidly. Collectively the results suggest that neem leaves extract may be used as an alternative approach to treat tick infestation where conventional producers are not available or contraindicated.

Keywords: Ivermectin, Neem, Tick and Calves

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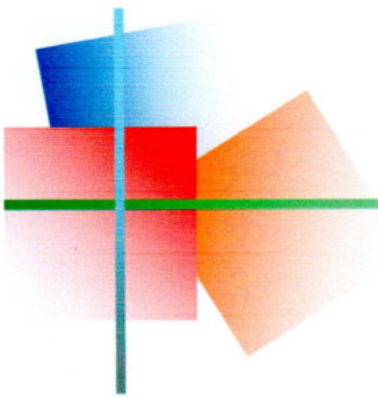
LIST OF ABBREVIATION AND SYMBOLS

ALP	-	Alkaline phosphatase
AE	-	Aqueous extracts
ALT	-	Alkaline aminotransferase
As	-	Arsenic
AST	-	Aspartate aminotransferase
BAL	-	British Antilewsite
BBS	-	Bangladesh Bureau of statistics
BER	-	Bangladesh Economic Review
b. w.	-	Body weight
cm	-	Centimeter
CNS	-	Central Nervous System
Conc.	-	concentration
cumm	-	Cubic millimeter
d.w.	-	Drinking water
ESR	-	Erythrocyte sedimentation rate
Fig.	-	Figure
gm	-	Gram
Hb	-	Hemoglobin
HSTU	-	Hajee Mohammad Danesh Science and Tecnology University
IP	-	Intraperitonial
kg	-	Kilogram
lit	-	litre
mg	-	Milligram
ml	-	Milliliter
mm^3	-	Cubic millimeter
No.	-	Number
PBS	-	Phosphate Buffer saline
PCV	-	Packed cell volume

LIST OF ABBREVIATION AND SYMBOLS (CONTD.)

ppm	-	Parts per million
rpm	-	Rotation per minute
S/C	-	subcutaneous
SGOT	-	Serum glutamate oxaloacetate transaminase
SGPT	-	Serum glutamate pyruvate transaminase
PCV	-	Packed cell volume
ppm	-	Parts per million
rpm	-	Rotation per minute
S/C	-	subcutaneous
SGOT	-	Serum glutamate oxaloacetate transaminase
SGPT	-	Serum glutamate pyruvate transaminase
-SH	-	Sulphhydral
TEC	-	Total Erythrocyte Count
Vol.	-	Volume
TLC	-	Total leucocyte count
µg	-	Microgram



Chapter 1

INTRODUCTION

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The economy of Bangladesh is mainly based on Agriculture. Our agriculture primarily depends on Livestock. Livestock is considered as the backbone of agriculture (Ahmed, 2000). In Bangladesh agricultural activities centre around crop cultivation which accounts for over two-thirds of agricultural value added. Another 15% of the value added originates from livestock and poultry rearing, which are activities supplementary to crop husbandry, carried out by using homestead land and surplus family labor of farm workers (Khan *et al.* 2004). Livestock provides new raw material for industry, serves a social security for the rural poor, and provides security against crop failure or damage during draught or cyclone. The contribution of the livestock sub-sector to GDP at constant prices was 1.84% in the fiscal year 2012-13 (BER, 2014). Cattle among other livestock species available are the most versatile component in relation to existing integrated agricultural farming system in Bangladesh. Tropical, agro-based Bangladesh has 53.21 million livestock of which 23.34 million are cattle (BER, 2014). Cattle are the main source of animal protein as they provide meat, milk and source of draft power and hides. From cattle, Bangladesh gets 173 thousand metric tons beef and 782 thousands metric tons milk per year. Further, the country gets 18 thousand metric tons butter and ghee and 32 thousand metric tons excellent type of hides from cattle (BBS, 2001). Bangladesh earns 3% foreign money by exporting leather and leather goods (EPB, 2011).

Cattle rearing in Bangladesh is hindered by various problem among them malnutrition and parasitic infestation are the major limiting factors (Jabbar and Green, 1983). Bangladesh is a moderately hot and humid country with short winter and prolonged rainy season and the geo-climatic condition of Bangladesh is suitable for the development and survival of various parasites as well as ticks (Islam *et al.* 2006). Tick infestation presents a serious challenge to farmers in both developed and developing countries (Jongejan, 1999).

Different species of ticks are widely distributed in Bangladesh and a number of researchers reported the distribution and abundance of tick species in different parts of the country (Rony *et al.*, 2010). They have a major direct effect on the husbandry and productivity of livestock, weight gain (Gibney *et al.*, 1985), milk production and quality of hide (Coles *et al.*, 2003). When present at high intensities, ticks may cause harm indirectly, such as nuisance, reduced time spent for grazing or ruminating, rubbing and self-wounding (Weeks *et al.*, 1995) and cause direct damage to skin and other sub-cutaneous tissues leading to alopecia and excoriation (Wall and Shearer, 1997). Also, ticks have been reported to transmit various diseases such as hemorrhagic fever, babesiosis, theileriosis, anaplasmosis etc (Roberts and Janovy 2005; Rajput *et al.,* 2006). Ticks act not only as potential vectors but also as reservoirs of certain infectious agents e.g., *Pasteurella multocida*, *Brucella abortus* and *Salmonella typhimurium* in man and animals (Jongejan and Uilenberg, 2004).

Chemical control of tick is the most widely used control strategy, but have major disadvantages like development of drug resistance in ticks (Hansens, 1956; Nolan and Schnitzerling, 1986; Nolan *et al.*, 1989; Schnitzerling *et al.*, 1989; Mendes *et al.*, 2001; Rodriguez-Vivas *et al.*, 2006; Li *et al.*, 2007; Miller *et al.*, 2007) and residual effects in animal products is another threat (Kaemmerer and Butenkotter, 1973).

Farmers and pastoralists have a long history of the use of traditional medicines and they have enough knowledge of their environment (Nfi *et al.*, 2001). Plant parts and their extracts have been, and still are used in many parts of the world to kill or repel insects (Secoy and Smith, 1983). More than 2400 plant species have been reported to have some pest control properties (Grainge and Ahmed, 1988) and this number is continuously increasing with the passage of time. A number of plants have also been identified for having anti-tick activity (Cruz-Vazquez and Ruvalcaba, 2000; Webb and David, 2002; Thorshell, 2006; Habeeb, 2010). Some of these plants are; *Azadirachta indica* (Benavides *et al.*, 2001), *Gynandropsis gynandra* (Lwande *et al.*, 1999), *Cleome hitra* (Ndungu *et al.*, 1999), *Pimenta*

dioica (Brown *et al.*, 1998) and *Tamarindus indica* (Chungsamarnyart and Jansawan, 2001).

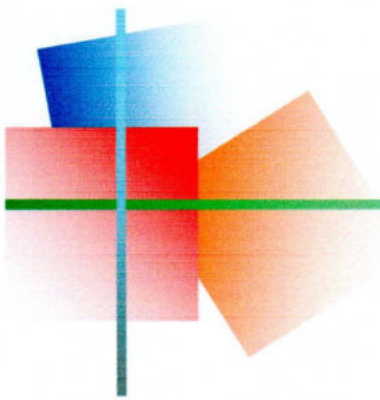
Generally, farmers in Bangladesh do not apply management programs to control tick infestation on their livestock. Although most of the commonly available insecticides are effective against ticks in Bangladesh, these are costly and fears of contamination of the environment and potential toxic effects on humans and other non-target organisms. Therefore, there is a need for alternative tick control measures that are effective, safe and economically and environmentally acceptable. Natural plant products would be suitable candidates. *Azadirachta indica* has proven to be effective against many insect pests and disease vectors of agricultural products (Wilps, 1986; Zebitz, 1986; Schmutterer, 1990) and is widely distributed in many parts of Bangladesh, especially in the dry areas where livestock farming is common. A number of compounds have been isolated from various parts of the tree, viz. meliantriol, salannin, azadirachtin and the triterpenoids, nimocinolide and isonimocinolide (Siddique *et al.*, 1986; Biswas *et al.*, 2002; Sharma *et al.* 2011). Neem extract seems to disrupt the mating and oviposition of insects and inhibits the hatchability of their eggs and moulting of their nymphs (Rembold *et al.*, 1986; Sazena, 1993). Additionally, in Bangladesh many drugs are being used for a long time to combat tick infestation in livestock. Due to indiscriminate use, ticks became resistant against the drugs.

The macrocyclic lactones are broad-spectrum antiparasitic drugs, extensively used in veterinary medicine. They are known as "endectocide" compounds based on their unique activity against endo-and ectoparasites (Shoop *et al.*, 1995). The macrocyclic lactons include two chemical families: avermectins (abamectin, ivermectin, doramectin, eprinomectin and selamectin) and milbemycins (nemadectin, moxidectin, d-milbemycin, etc.), which are commercially available to use in livestock and pet animals as injectable, oral and/or pour-on formulations (McKellar and Benchaoui, 1996). Ivermectin is a member of the macrocyclic lactone class of endectocides, commonly referred to as avermectins. It is used for the treatment of internal and external parasites in dogs, cats, horses, pigs, sheep

and cattle. It is effective against number of parasites such as nematodes, acarins and insects, and it also improves the skin coat of animal (Negrea 1997). Subcutaneous (SC) and topical (TOP) formulations are available for use in cattle, at a dose of 0.2 and 0.5 mg/kg bodyweight (BW), respectively. Ivermectin is undoubtedly the most revolutionary parasiticide in modern veterinary medicine, the one with the broadest spectrum of activity, high efficacy as well as a wide margin of safety and the most sold. It is prepared from abamectin, a natural fermentation product of soil bacterium *Streptomyces avermitilis* (Payne *et al.* 1995). Ivermectin is safe and effective against both ecto and endo parasite of animals but it is expensive. In contrast, the alternative cheapest and available source of drug is herbal therapy. Experimental investigations, therefore, is imperative to assess the therapeutic value of indigenous herbal plants and leaves. In vivo research work in this field is yet very limited in our country.

Therefore, the present research work was undertaken with the following objectives:

I. To investigate the efficacy of neem leaves extract (spray) and ivermectin (subcutaneously) against tick infestation in calves.
II. To study the effects of neem leaves extract (spray) and ivermectin (subcutaneously) on some clinical parameters in calves.
III. To study the effects of neem leaves extract (spray) and ivermectin (subcutaneously) on some hematological parameters in calves.
IV. To evaluate the therapeutic value of neem leaves against ectoparasites in calves.



Chapter 2

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

In spite of extensive development of synthetic drugs of the modern world, medicine is still depends upon plant kingdom. Some herbal drugs, such as belladona, opium and digitalis have no satisfactory substitutes. Men used the roots, leaves and bark of plants as the main source of plants as the main source of drugs for the treatment of various diseases. Among these diseases parasitic infestation is most important. The plants which are effective against parasites are Tobacco, Neem, Pineapple etc. The researchers have reported that they have moderate anthelmitics activity. In 19^{th} century, with the advancement of knowledge of the scientists, the active principles of the medicinally useful herbal drugs were identified by different methods of extraction. Alkaloid, glycosides and volatile oils obtained by extraction were found to be very important constituents out of many other constituents of plants such as Saponin, Resins etc. (Claus, 1962) considering the pharmacological activities of these extracts.

Although most of the commonly available insecticides are effective against livestock ticks in Bangladesh but they have several disadvantages including high costs, contamination of the environment, toxicity to man and animals as well as other off-target organisms. Alternative tick control methods that are effective, save, economical and socially acceptable and environmentally friendly, are therefore required. Various natural products including plants could be suitable candidates for such an alternative approach. Among the plants, *Azadirachta indica* (Neem plant) has been shown to be potentially larvicidal against ticks, fleas and other insects. It has also been proven to be effective against many insect pests of agricultural products and disease vectors (Schmutterer, 1981; Zebitz, 1986; Wilps, 1986).

Now a days, there are lots of anthelmintic are available in the market, of them Ivermectin is important. The avermectins probably represent the biggest breakthrough in parasitic control since the discovery of the Benzimidazoles in the

early 1960s. Avermectins and the closely related Milbemycins are antibiotics produced by actinomycete microorganism and are termed macrocyclic lactones (or macrolides). They are highly potent and show activity against a wide range of parasites from nematodes to ectoparasitic arthropods. The drug required in a very small dose (microgram quantities instead of milligrams) and may be administered orally or parenterally, their activity persists for long time after dosing. There are eight major avermectins derived from the organism *Streptomyces avermitilis* and will over 30 milbemycins from a number of related *Streptomyces* spp.

2.1 Neem

Scientific name: *Azadirachta indica* Linn

Synonyms: Melia azadirachta Linn.; Melia indica, Brandis

Family: Meliaceae

English: Neem / Nim, Margosa, Indian Lilac

Neem (*A. indica*) tree is used in reforestation projects in hot, dry regions. In this century knowledge about the tree has been spread to the west, where it has been hailed as a "wonder plant". Neem based pesticides have been developed and the potential health uses of chemical extracted from the tree are being studied. It has been introduced and established throughout the tropics and subtropics for its highly valued hardiness, it's almost year-round-shade and its multiple wood and non-wood products.

Description

Neem is a large, evergreen perennial tree, 15-20 m in height with a straight trunk; leaves are simple, numbers of leaflets are 9-15, opposite or alternate, lancet, acuminate or sub falcate. Flowers are white, Drupe is oblong. The wood resembles mahogony and the bark is very bitter (Joshi, 2000).

Distribution

A common tree of Bangladesh and throughout the greater parts of India, planted in the hot climate. Neem in different areas and 28 languages is known by different name such as neem, nim (Hindi and Bangla), timba (Gujrate), bevu (Kannada), vepe (Malaylam) and vepa (Tamil).

Chemical constituents

Various parts of the plant and the neem oil contain triterpenoid bitter principles, saponins, flavonoids, tannins and alkaloids. The bitter principles include nimbidin, nimbin, nimbinine, 6-desacetylnimbinine, nimbidol, nimbolide, quercetin and its glycosides, beta-sitosterol, hexacosanol, nonacosane, ascorbic acid and amino acids. Barks contain nimbolins A, B, organic acids, tannin, margosin and azadarin. Flowers contain essential oil, kaempferol, kaempferol glucoside, nimbosterin and N-nonacosane. Fruits contain resins, tannins, triterpenoids, salanin and azadirachtin, melianone, oil and organic acids. Seeds contain six tetranortriterpenes and four new limonoids, 11 hydroxy azadirachtin B, 1 tigloyl 3 cetyl azadirachtin, 1, 2 diacetyl 7 tigloyl 12 hydroxy lvilasinin and 23 desmethyl-limocin-B. Neem oil contains margosic acid. The isolation and structure elucidation of the antimalarial agent of the plant, gedunin, has been reported in 1989 (Ghani, 2003).

Uses

Neem is used in Ayurvedic for leprosy and skin diseases, fever and purification of blood. Every part of the plant is used medicinally. Various part of the plant are used to treat gingivitis, sores, fevers (including malaria), spleen complaints, tumours, head scald small pox, diarrhoea and cholera. In addition, the leaves possess antiseptic properties and are used in boils, ulcers, eczema, ringworm and scabies. Aqueous and alcoholic extracts of the leaves and bark show good antibacterial activity. Extracts of leaves, bark, gum and seeds are used as remedies for scorpion-sting, snake-bite, as an antiviral, antineoplastic and antifungal agents. The gum is a demulcent tonic and is useful in catarrhal affections. Flowers are

used in atonic dyspepsia and general debility. Seed kernel reduces anti-diabetic and anti-hyperlipaemic effects in alloxan diabetic rabbits. The oil is used in the treatment of ulcers, chronic skin diseases and rheumatism. Most constituents of the plant exhibit antibacterial and ant-inflammatory effects (Ghani, 2003).

The neem oil serves as a useful local remedy in some chronic forms of skin disease and ulcers by stimulating and exciting a healthy action. The oil is also extremely useful as an anti-parasite in various cutaneous afflictions, such as ringworm, scabies and others where the presence of any kind parasite may be suspected. It rapidly destroys the parasite and induces a healing reaction when the parasite is in the deeper layers of the skin, it will be necessary to rub the oil for 10 minutes or more at a time (Kritikar and Basu, 1918). The oil is used as a repellent against pests in cattle sheds. Neem oil is widely used in the treatment of livestock. The bitter property and its characteristic smell made it a good and cheap wound dressing by keeping away flies (Perera, 1941). *A. indica* is a homeopathic remedy. It is a grand remedy in chronic fever.

A lot of researches have done on ticks and many products have now been recognized as potent acaricides all over the world. There are many reports published on the use of plant materials against tick infestation. Several species of ticks have been reported to be controlled and treated applying botanical products in the form of powder, extract and oil as potential acaricide. Very limited in vivo research has been conducted on the use of medicinal plants in Bangladesh. In this chapter relevant information on the research works on ticks as well as treatment and / or control of ectoparasites by botanical acaricides have been cited.

Peixoto *et al.* (2013) carried out an experiment to evaluate the citronella oil and neem oil in the control of bovine ticks. Through the technique of adult ticks immersion, 280 ticks were evaluated. They were distributed to equal number throughout four treatments: negative control group, positive control (ivermectin), neem oil and citronella oil. The mortality index, estimated reproduction, product

efficiency, eggs production index and hatchability rate were analyzed. The efficiency of the product, in terms of mortality index just, was 100%, 97.14% and 82.86% in control group, citronella oil and neem oil, respectively.

Zaman *et al.* (2012) studied the anti-tick efficacy of combined aqueous herbal extracts of *Azadirachta indica* leaves, *Nicotiana tabacum* leaves, *Calotropis procera* flowers and *Trachyspermum ammi* seeds using adult immersion test, larval packet test and ear bag method. The extract exhibited lethal effects on egg laying (index of egg laying =0.371404±0.00435), hatching (22.35%) and total larval mortality at 50mg/ml and reduced tick intensity on the infested calves (18 detached out of 35 at 45% (w/w) suspension, topically applied). The herbal extract exerted dose- and time-dependent effects against all the developmental stages of *Rhipicephalus* (*Boophilus*) *microplus*.

Giglioti *et al.* (2011) studied *in vitro* acaricidal activity of neem (*A. indica*) seed extracts with known azadirachtin concentrations against *Rhipicephalus* (*Boophilus*) *microplus*. The effect of four extracts from neem seeds (*Azadirachta indica*) containing 2000, 5000, 9000 and 10,000 ppm of azadirachtin A (AZA), quantified by high performance liquid chro-matography (HPLC) and diluted to 1.25%; 2.5%; 5.0%; 10.0% and 12.8% was verified by *in vitro* tests with engorged females and larvae of the cattle tick *R. microplus*. The results from the bioassays with the engorged females showed that the main toxic effect of the extracts was reduction of the reproductive parameters, with a sharp drop in the number of eggs laid and the hatching rate, mainly when the extracts were diluted to 10.0% and 12.8%. The product effectiveness (PE) calculations for all the solutions tested showed that the AZA solution at 10,000 ppm (N10) was the most effective. However, larvae showed zero effectiveness of all the extracts tested. The results of the tests with engorged females showed that the neem extracts had acaricidal activity, inhibited egg laying and reduced hatchability rate.

Schmahl *et al.* (2010) summarized the acaricidal and insecticidal effects of a patented neem seed extract when diluted 1:10 with shampoo or 1:20, 1:30, 1:33, 1:40, respectively, 1:66 with tap water. It was shown that a broad range of pests

and parasites, such as *Ixodes* and *Rhipicephalus* ticks, house dust mites, poultry mites, harvest mites, cat fleas (adults, larvae), bed bugs (all stages), head lice and mallophaga, cockroaches (genera *Blatta*, *Blattella*, *Gomphadorhina*), raptor bugs (*Triatoma*), and even food-attacking beetle (*Tenebrio molitor*) might be controlled with this extract. Tests on skin compatibility proved that there are no skin irritations during or after use.

Xu *et al.* (2010) studied the acaricidal activity of preparation of neem oil microemulsion *in vitro*. They found that 10% neem oil microemulsion killed *Sarcoptes scabiei* var. *cuniculi* larvae by 192.5 min *in vitro*. The median lethal time value was 81.7463min with the toxicity regression equations of Y=−6.0269+3.1514X. These results demonstrated that neem oil microemulsion was effective against *Sarcoptes scabie* var. *cuniculi* larvae *in vitro*.

Choudhury (2009) conducted an experiment on the toxicity of Neem Seed Oil against the larvae of *Rhipicephalus* (*Boophilus) decoloratus*, a one-host tick in cattle. The *in vitro* efficacy of neem seed oil was tested against the larvae of a one-host tick, *Boophilus decoloratus* parasitic mainly to cattle generally found in savannas of tropical equatorial Africa. The 20, 40, 60, 80 and 100% concentrations of neem seed oil were found to kill all (100% mortality) the larvae after 27, 27, 27, 27 and 24 h respectively.

Srivastava *et al.* (2008) prepared extracts from leaf, bark, and seed of *Azadirachta indica*, leaf and seed of *Prunus persica*, bark of *Mangifera indica*, and leaf of *Psidium guajava* and were evaluated against *Rhipicephalus* (*Boophilus) microplus*. Of the eight extracts screened, the extracts prepared from the *A. indica* seed showed very high level of efficacy (80%) after 5 h of treatment. Besides the immediate effect on adult ticks, the egg-laying properties of the survived ticks was also assessed, and a significant reduction ($P < 0.01$) in the reproductive index of ticks fed on animals treated with *A. indica* seed extracts was noted in comparison to control. The efficacy of the neem seed extracts was compared with the commonly used synthetic pyrethroids, and comparable efficacy against *R. microplus* fed on animals treated with neem seed extracts and acaricide

treated was noted. They suggested the possibility of using the extracts in IPM format for the management of ticks.

Tabassam *et al.* (2008) conducted a study which aimed to evaluate the efficacy of crude aqueous-methanol and aqueous extracts of neem seed kernel against sarcoptic mange in sheep. They prepared crude aqueous-methanol (AME) and aqueous extracts (AE) of neem seed kernel (NSK) and formulated as 10% and 20% ointments (w/w), using vaseline as vehicle. Ivermectin (positive control) completely cleared infesting mites from animals after 10 days and 20% AME after 16 days. While, clinical mange was completely cured after 16 and 20 days with ivermectin and 20% AME, respectively, under field conditions.

Habluetzel *et al.* (2007) studied the secondary metabolites present in the neem tree exhibit a wide range of biological activities against insects. They studied the efficacy Neem Azal, an azadirachtin-rich extract of neem seeds, in controlling *Damalinia limbata* (Phthiraptera) louse infestation in angora goats in Central Italy. Groups of 11-12 goats were treated with Neem Azal at an azadirachtin concentration of 650 ppm or 125 ppm, with Neguvon or were left untreated. A reduction in louse densities of 76-96% was observed from week 2 to week 18 after treatment with the neem solution containing azadirachtin at a concentration of 650 ppm. They observed 60-92% reduction of louse from week 2 to week 14 at the lower test concentration (125 ppm). Neem Azal was found to reduce the survival of both adult and nymph stages of *D. limbata* and to interfere with oviposition and oogenesis of female lice. Their ovaries revealed morphological alterations in both vitellogenic and previtellogenic ovarioles at the follicular and germinal level. Since neem compounds target different life stages and physiological processes of *D. limbata*, the development of insecticide resistance by biting lice exposed to neem-based insecticides appears unlikely. For this reason and for its prolonged activity, which in principle allows angora goats to be protected for a large part of the mohair production cycle, neem-based insecticides may have a potential interest for mohair producing breeders.

Kumar *et al.* (2005) conducted an experiment on the efficacy of ivermectin and neem with Karanj oil against natural *R. microplus* infestation in cattle. Single dose of ivermectin subcutaneously @ 200 µg kg $^{-1}$ body weight showed 100% efficacy against *R. microplus* ticks on day 8 post-treatment in naturally infested cows and remained so up to day 25 of application. A 50:50 mixture spray of neem + karanj oil achieved 97.95% freedom from natural tick infection in cows on day 15 post application suggesting that formulation is a safe alternative to control ticks in field conditions.

Shivastava and Das (2003) selected 58 cattle infested with *Rhipicephalus (Boophilus) microplus* in Beraghar, Chhattisgarh, India and divided into 6 groups. Six plant oils, namely *Cucurbita maxima*, *Terminalia arjuna*, *Azadirachta indica*, *Pongamia pinnata*, *Sapindus trifoliatus* and *Ricinus communis*, were applied on different groups of cattle. Application of the oil for 72 h showed good response in all treatments. The percentage of tick control was highest for ritha oil (78.58%), followed by karnaj oil (62.22%), neem oil (52.46%), kahua oil (45.77%), kaddu oil (39.22%) and castor oil (22.45%).

Abdel-Shafy and Zayed (2002) conducted an experiment on *in vitro* acaricidal effect of plant extract of neem seed oil on eggs, immature, and adult stages of *Hyalomma anatolicum excavatum* (Ixodoidea: Ixodidae). Effects of the plant extract of neem seed on eggs, immature, and adult stages of *H. anatolicum excavatum* was studied at concentrations of 1.6, 3.2, 6.4, and 12.8%. The extract was found to have a significant effect on the hatching rate of eggs. It significantly increased the hatching rate during the first 7 days post-treatment (DPT) giving incompletely developed and dead larvae; however, it cause hatching failure at DPT 15. Neem Azal F induced a significant increased in mortality rates of newly hatched larvae, unfed larvae, and unfed adults reaching 100% on 15th, 3rd, and 15th DPT, respectively. The mortality rates increased with the extract concentrations. Although, it had no significant effect on the moulting rates of fed nymphs, it caused malformation or deformities in 4% of adults moulted.

Webb and David (2002) evaluated the use of Neem seed extract for controlling common cattle ticks. Three bulls and three cows of the Tswana, Brahman and Simmenlaler breeds were cleared of ticks using ether and hand picking techniques. Each animal was taken naturally infested with ticks. A 5% (w/v) water extract of neem seed kernel was applied at a rate of 5 gm/kg body weight to various anatomical sites on the animals selected for the experimental treatment, while the control treatment animals were sprayed with tap water. Treated and control animal grazed together and were mustered at weekly intervals for examination. Tick population densities on animals treated with neem seed extract were lower than on untreated animals. Indigenous Tswana cattle harbored fewer ticks during periods of tick abundance than Brahman or Semmentaler cattle. It was concluded that neem seed extract is effective in controlling ectoparasites on livestock.

2.2 Ivermectin

Chemistry:

Ivermectin (CAS-7-288-86-7) is a mixture of two compounds belonging to a class of substances known as avermectins. The chemical names are 5-0-demethyl-22, 23-dihydroavermectin A_{1a} and 5-0-demethyl-22, 23-dihydroavermectin-A_{1b}. These are also known as 22, 23-dihydroavermectin B1a and 22, 23-dihydroavermectin B_{1b}. Ivermectin contains at least 80% of 22, 23-dihydroavermectin B_{1a} and less than 20% of 22, 23-dihydroavermectin B_{1b}. The avermectins are derivatives of pentacyclic sixteen-membered lactones. Within the family of the avermectins, there exist two series, A and B, within which are two structural subsets, designated c1 and 2, consisting of two homologs a and b. members of the A-series are methoxylated at the carbon atom in positive five, whereas the B-series compounds have an underivatized hydroxyl-group at this position. Compounds of the 1-subset possess an olefinic bond between the two carbon atoms C_{22} and C_{23}; this double bond is hydrated in the 2-subset, resulting

in a hydroxyl group at position 23. This difference has profound effect on the conformation of the ring bearing these functionalities and causes subtle changes in bioactivity (Chabala *et al.*, 1980).the a- and b- homologs differ by their substituents at position 25, with a-homologs having an isopropyl group, derived from L-valine, and b-homologs possessing a sec-butyl group derived from L-iso-leucine during biosynthesis. Avermectins are glycosides with a disaccharide attached to the hydroxyl group at C_{13}. The two identical sugars have been identified as L-oleandrose, a dideoxymethyl-aldohexose.

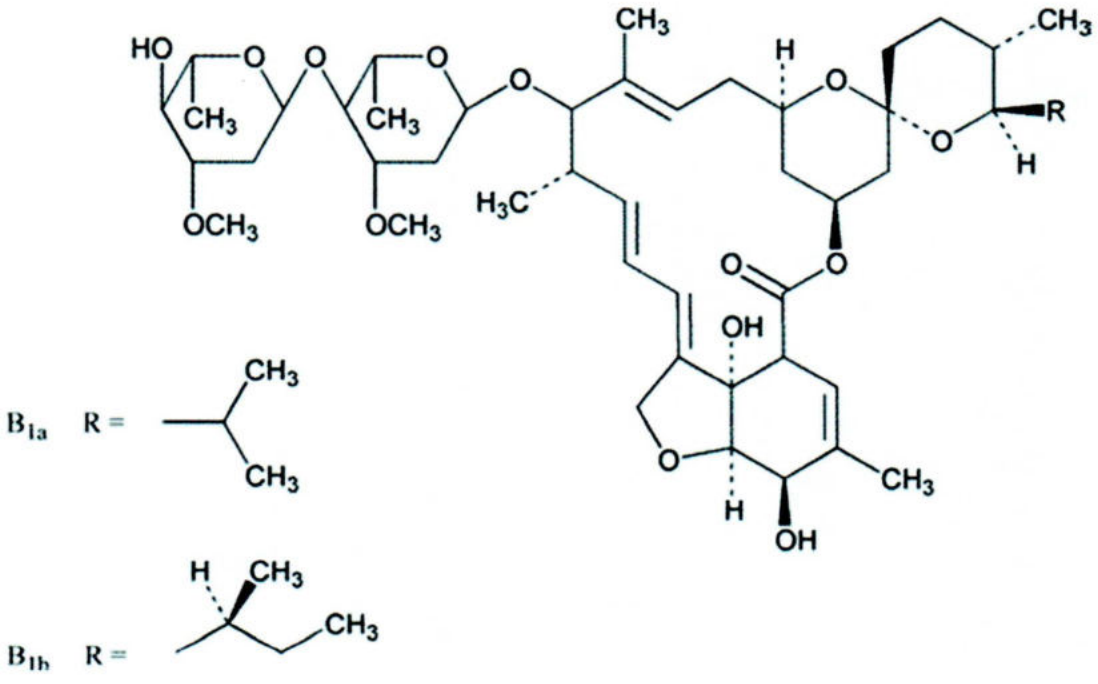


Fig 1: Structure of 22, 23-dihydroavermectin B_{1a} and 22, 23 dihydroavermectin B_{1b}.

Ivermectin (as its major component, 22, 23-dihydroavermectin B_{1a}) is an off-white powder that is highly lipophilic and hydrophobic. It dissolves in most organic solvents but is poorly soluble in water. It is stable at room temperature in non-acidic solutions but is degraded by UV light.

Mode of action

It was originally thought that the macrolide endectocyides increased the release of gamma-aminobutyric acid (**GABA**) from synaptosomes of the nervous system. This, in turn, opened GABA-gated chloride channels. It is now known that these compounds open chloride channels in invertibrates via a specific binding site that is glutamate-gated, although the binding site apparently occurs in close anatomic proximity to GABA-gated sites and the macrolide endectocides may potentiate GABA-gated sites as well. About 50 percent of the effect of a macrolide endectocide can be reversed with picro toxin, a GABA antagonist active at the chloride channel. In nematodes, the synapse between inter-neurons and excitatory motor neurons is the primary site of action, whereas the myoneural junction is the primary site in arthropods. In either case the chloride ion influx lowers cell membrane resistance and causes a slight hyper polarization of the resting potential of postsynaptic cells. This makes neurotransmission more difficult so that transmission of stimuli to muscles is prevented, resulting in a flaccid paralysis of affected parasites followed by their death or expulsion.

Pharmacokinetics

Pharmacokinetics studies with ivermectin are summarized by Fink and Porras (1989). The specific formulation used, the route of administration and the animal species to which it is administered affect the pharmacokinetics of ivermectin. The biological half-life ($t_{1/2}$) of ivermectin in plasma following IV administration of 300 µg /kg to cattle is 2.8 days. IV administration to sheep gives a similar biological half-life ($t_{1/2}$ = 2.7 days) to that in cattle but a lower plasma concentration due to a greater volume of distribution in sheep than in cattle (1.9 vs. 4.61 / kg). Ivermectin is eliminated more rapidly in dogs ($t_{1/2}$ = 1.6-1.8 days).

Subcutaneous administration of the commercial formulation of ivermectin to cattle at a dose rate of 200 µg / kg results in a longer biological half-life ($t_{1/2}$ = 8 days) than IV administration due to slow absorption from the injection site. A peak plasma concentration (C_P) of 44 mg /ml occurs at 2 days after S/C injection.

Clinically significant anthelmintic efficacy persists for approximately 2 weeks for pour-on formulation depending upon parasite species. Oral dosing in sheep results in a $t_{1/2}$ of 3-5 days.

Following administration, ivermectin residues are lowest in brain and highest in liver, bile and fat (Chiu and Lu, 1989). Depletion half-lives were 4.8 and 7.6 days for liver and fat, respectively, in cattle and sheep. Tissue redistribution patterns are similar for cattle, swine and rats, but depletion half-lives for liver and fat are shorter in sheep and rats than in cattle or swine. The parent drug is the major liver residue for 3, 5, 7 and 14 days after dosing in rats, sheep, swine and cattle respectively.

Fecal excretion is the main route of elimination, accounting for 98% or more of excreted ivermectin, with the remainder appearing in the urine, except upto 5% of the dose may be excreted in the milk of lactating animals.

Anthelmintic spectrum in cattle

In cattle, ivermectin is effective against the major gastrointestinal (e.g., *Haemonchus* spp, *Cooperia* spp, *Ostertagia* spp, *Trichostrongylus* spp) and pulmonary (e. g., *Dictyocaulus* spp) parasitic roundworms. It is also effective against most tick (e. g., *Rhipicephalus microplus*), mites (e. g., *Psoroptes ovis*, *Sarcoptes scabiei* var. *bovis*) and lice (e. g., *Linognathus vituli*, *Haematopinus eurysternus*, *Solenopotes capillatus*) species and against numerous myiases (e. g., those caused by screw worm, bot flies and warble flies).

Vega *et al.* (2013) evaluated the effect of three ectoparasiticides —cipermetrin, amitraz, and ivermectrin on *R. microplus*. After egg hatching, a hundred larvae of 14-21 days old, were sampled on 2 x 2 cm filter paper strips upon the larvoscopic examination using impregnated filter paper. Every ectoparasiticide was tested for its effect on each sample in a 1 ml dose and increasing concentrations of 0.05 %, 0.125 %, 0.250 %, and 0.5 %; whereas the control group was administered castor oil. The larvae under medication in the trials remained 24 h in the dark at room temperature, followed by a living-and-dead specimen counting. Acarid likelihood

mortality percentage associated with ectoparasiticide kind and concentration variables was estimated by the Probit Regression technique. Treatment was less effective for cipermetrin (37.1 %), rather than amitraz and ivermectrin (89.9 % and 73.3 %, respectively) at similar concentrations.

Manjunath and Kumar (2012) compared the efficacy of flumethrin and ivermectin on 50 Deoni bullocks infested with ticks (*R. microplus*). The severity of the tick infestation was determined on a four point scale and were grouped into three classes *viz*., heavy infestation: 5-6 ticks/sq. inch (+++), moderate infestation: 3-4 ticks/sq. inch (++), and mild infestation: 1-2 ticks/sq. inch (+). They found that the bullocks were heavily infested with ticks (+++) at the beginning of the experiment. They divided animals into two treatment groups each consisting of 25 animals. Group A animals were administered flumethrin topically @ 1 ml/10 kg body weight. Group B animals were administered ivermectin subcutaneously @ 200 µg/kg body weight. They observed animals for the density of ticks on 0, 3, 10, 30 and 45th day of treatment. In addition, they collected blood samples from the animals on 0th and 30th day, and analysed for haematological parameters *viz*., blood Hb, PCV and RBC count. Treatment completely removed ticks from the body surface of the animals treated with ivermectin on the next day of treatment. Similar situation was observed on the 20th day of treatment in the group treated with flumethrin. The animals treated with ivermectin were completely free from ticks with one treatment, whereas ticks appeared on the body surface of the animals (++) treated with flumethrin on the 45th day of treatment. There was significant ($P \leq 0.01$) improvement in Hb, PCV and RBC count in the ivermectin treated group on 30th day, while the haematological picture remained unchanged ($P \geq 0.05$) in the flumethrin treated group, suggesting ivermectin was a highly effective acaricide against tick infection in cattle. A single injection of ivermectin provided instantaneous relief to the animals from tick infestation with complete restoration of haematological indices. There was no recurrence of infection.

Santana *et al.* (2012) evaluated different acaricide treatments for the control of *R. microplus* on field-kept dairy cattle in the state of Pernambuco, Brazil. The first

phase of the experiment consisted of collecting the ingurgitated female *R. microplus* directly from the hosts for attainment of larvae. Then, they infested cattle with larvae. They separated animals into 12 groups to receive the corresponding treatment: Abamectin; Ivermectin; Ivermectin LA; Amitraz; Amitraz + Ivermectin; Amitraz + Ivermectin LA; Amitraz + Abamectin; Association (Cypermethrin + Chlorpyrifos + Citronella); Association + Ivermectin; Association + Ivermectin LA; Association + Abamectin; and Control. Subsequently they evaluated on post-treatment days +7, +14, +21, +28, +35, +42, +49, +56 and +63. Indices revealed considerable variation ranging from 0% to 96.63%. Such indices demonstrate the significant reduction in the number of ticks on the animals in some groups, especially in the abamectin group. They found that the use of different avermectines can assist in the development of *R. microplus* control programs, thereby reducing the number of acaricide applications and production costs related to ticks.

Ram and Sharma (2010) evaluated the therapeutic efficacy of ivermectin and doramectin (S/C injections) and flumethrin (pour on preparation) against natural *R. microplus* infestation in crossbred cattle. Tick mortality observed on seventh day post treatment in different groups indicated 95, 96 and 97% efficacy of ivermectin, doramectin and flumethrin, respectively. However, residual drug effect (duration of protection) for ivermectin and doramectin injections was recorded less than 21 days in comparison to flumethrin pour on (28 days). Further, they concluded from the study that a second follow up treatment on or after third week in case of ivermectin and doramectin (S/C injections) and after fourth week in flumethrin (pour on) medication is required for proper control of ticks in endemic areas.

Davey *et al.* (2010) determined concentration-time profile, therapeutic and persistent efficacy of a single subcutaneous injection of cattle with a long-acting (LA) formulation of ivermectin at a concentration of 630 µg per kg of body weight against *R. microplus*. Ivermectin sera concentration increased to 13.0 ppb within 1 d after treatment and peaked at 26.4 ppb at 11 d post-treatment.

Ivermectin levels remained above the threshold level for control of feeding ticks (equal to or greater than 8 ppb) for 42.6 d after treatment. Therapeutic efficacy was > 99.9%, and tick number, index of fecundity and fertility, engorgement weight, and egg mass weight of treated ticks were dramatically less than those of the untreated group. The persistent efficacy indicated tick number and reproductive capacity of ticks infested on treated animals at 14 and 28 d post-treatment were less than untreated ticks, whereas engorgement weight and egg mass weight remained lower than that of untreated ticks 49 d post-treatment.

Hussain *et al.* (2005) conducted an experiment for chemotherapeutic control of bovine pediculosis. For chemotherapeutic trial, thirty cattle and buffaloes each were randomly divided into three major groups (A thru C) each of which was further divided into two sub groups for cattle and buffaloes, respectively. The major group A was treated with ivermectin (S/C 200µg/kg b.w.) and group B was treated with topical application of cypermethrin (1ml/200ml water). The animals of group C1 were given a sham treatment of propylene glycol (S/C 8-10 ml/large animal) whereas the topical application of normal saline was done on the animals of group C2. Number of lice per animal before and after treatment was counted to determine the efficacy of drugs. It was observed that maximum control (100%) was achieved on day 28 post-treatment with the ivermectin-treated buffaloes.

Davey *et al.* (2005) studied therapeutic and persistent efficacy of a single injection treatment of ivermectin and moxidectin against *R. microplus* on infested cattle. The effectiveness of a single treatment with either ivermectin or moxidectin was determined by administering a single subcutaneous injection of each endectocide at 200 µg per kg b. w. to cattle infested with all parasitic developmental stages (adults, nymphs, and larvae) of *R. microplus*. They found that ivermectin and moxidectin killed 94.8 and 91.1% ticks respectively. In addition, the reproductive capacity of the females that did survive was reduced by >99%, regardless of the endectocide. Based on these two factors, the therapeutic level of control obtained against ticks on the cattle at the time of treatment was 99.0 and 99.1% for ivermectin and moxidectin, respectively. Engorged females

recovered from either group of treated cattle weighed ≈3-times less than untreated females, and the egg masses produced by treated females weighed ≈5–8-times less than egg masses produced by untreated females. Partitioning of data into three separate 7-d post-treatment intervals allowed for an estimation of the efficacy of each endectocide against each individual parasitic development stage (adult, nymph, and larva). They found both endectocides were ≥99.7% effective against ticks.

Hanif *et al.* (2005) carried out research from February to March 2005 on 20 sheep of both sexes aged upto 3 to 4 years at the animal house of Veterinary Clinic, Bangladesh Agricultural University, Mymensingh to study the efficacy of ivermectin pour on against ectoparasites and its effect on certain hematological (Hb, TEC and PCV) parameters and b.w. gain. Fifteen sheep heavily infested with different ectoparasites were randomly divided into 3 equal groups (groups B, C and D) consisting of 5 sheep in each. Another 5 sheep free from ectoparasitic infestation were kept as uninfested control group (group A). Topical ivermectin preparation was administered at the dose rate of 400 µg/kg b.w. (pour on) and 500 µg/kg b. w. (pour on) in groups C and D respectively whereas sheep of group B was kept as infested control. The therapeutic efficacy of ivermectin was 100% against ectoparasites (lice and tick) after 7 days of treatment. The mean b. w. of the sheep of treated groups (C and D) was increased after treatment with ivermectin, on day 28 post treatment. Similarly, total erythrocyte count (TEC) and hemoglobin (Hb) content was increased significantly. However, PCV was also influenced appreciably.

Aziz *et al.* (2004) studied the comparative efficacy of ivermectin and diazinon against ectoparasites, their effects on some clinical, hematological and biochemical parameters in sheep. For this purpose 25 sheep heavily infested with ticks and lice were randomly divided into 5 equal groups, i.e. groups B, C, D, E and F. Another group of 5 sheep free from parasitic infestation were also selected and kept as uninfected control (group A) and group B was kept as infected control group. The sheep of groups C and D were treated with recommended (200 g/kg

b.w.) and higher than recommended (300 g/kg b.w.) doses of ivermectin subcutaneously. The sheep of groups E and F were treated with diazinon (spray) at recommended (0.1% soln.) and higher than recommended (0.2% soln.) doses. After injection of ivermectin and spray of diazinon all groups were obseved for 28 days and clinical, hematological and biochemical parameters were evaluated at 7 days intervals. The data were collected and analyzed statistically between control and treated groups by using student 't' test . On the basis of lice and tick count, the efficacy of ivermectin was found 100% whereas diazinon was 82-85% effective against ectoparasitic infestation in sheep. Ivermectin and diazinon increased the feeding efficiency as compared to infected control groups. No adverse effects was observed following both the dose of ivermectin and recommended dose of diazinon. In this study, the hematological parameters, i.e. TEC and Hb% were increased significantly ($p<0.01$) in all treated four groups to the extent of 35-70% and 10-21% respectively. On the other hand, ESR values were decreased significantly ($p<0.05$). No significant change was observed on SGOT and SGPT following both the doses of ivermectin injection. However, SGOT and SGPT values were significantly ($p<0.01$) increased to the extent of 46-90% and 25-46% within 7-14 days of diazinon spray. However, elevated values of SGOT and SGPT became almost normal within 28 day of treatment. Among the recommended and higher than recommended doses of ivermectin and diazinon, the recommended dose of both the drugs is suitable for therapeutic purpose. Among the two drugs, ivermectin was found to be the best drug against ectoparasites showing prompt and 100% efficacy.

Lifschitz *et al.* (2003) compared the plasma concentration profiles of four randomly chosen ivermectin (IVM) generic formulations (IVM G1–G4) after their subcutaneous (SC) administration to healthy calves. The disposition of other avermectin-type endectocide compounds, doramectin (DRM) and abamectin (ABM) was also assessed in the same pharmacokinetic trial. Forty-two parasite-free Aberdeen Angus male calves were randomly allocated into six treatment groups. Animals in each group *(n = 7)* received SC treatment (200 µg/kg) with one of the commercially available endectocide formulation used in the trial. Blood

samples were collected and analysed by HPLC with fluorescence detection. Large kinetic differences were observed among the DRM, ABM and IVM formulations. The DRM plasma concentration profiles were higher than those measured for ABM and all the IVM generic formulations. The higher and sustained plasma concentrations of DRM accounted for greater area under concentration–time curve (AUC) and longer mean residence time (MRT) values compared to those obtained for both ABM and the IVM generic preparations. The pattern of IVM absorption from the site of subcutaneous administration showed differences among the generic formulations under evaluation. The IVM G2 preparation showed higher peak plasma concentration and AUC values *($P < 0.05$)* compared to those obtained after the administration of the IVM G1 formulation. Longer *($P < 0.05$)* MRT values were obtained after the administration of the IVM G3 compared to other IVM generic preparations. The kinetic behaviour of ABM did not show significant differences with that described for most of the IVM formulations. This study demonstrates that major differences on drug kinetic behaviour may be observed when using different endectocide injectable formulations in cattle.

Islam *et al.* (2003a) conducted an experiment to determine the efficacy of ivermectin against gastrointestinal nematodes and ectoparasites in calves. A total of 20 calves having both G.I. nematodes and ectoparasites were selected and randomly divided into two groups namely group A (n=1 to 15 as treatment group) and group B (n=16 to 20 as control group). Ivermectin (subcutaneous formulation) was administered at the neck region of the calves in group 'A' at the dose rate of 200mg/kg b. w. The animals of group 'B' were kept as control. On the basis of faecal egg count, reduction on 5th, 10th, 15th, 20th, 25th day post-treatment the efficacy of ivermection was found 100% against G.I. nematodes infection of calves, 100% efficacy against lice infestation at 15th, 20th, 25th day post-treatment. Body weight increased 6.663% in calves after the treatment with ivermectin. A significant increase on the Hb was observed in the treatment group on 20th and 25th day post-treatment. The PCV level increased significantly from

the 15th to 25th day post-treatment. In treatment group, no side effects were observed.

Islam *et al.* (2003b) reported that ivermectin (Ivomec®) injection was 100% effective against natural infection of gastrointestinal nematodes in goats. TEC, Hb and PCV of treated goats were significantly increased whereas ESR values were decreased.

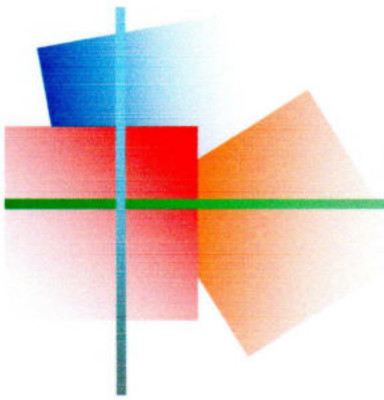
Davey and George (2002) studied the efficacy of Macrocyclic Lactone Endectocides against *R. microplus* infested cattle using different pour-on application treatment regimes. The efficacy of pour-on formulations of three macrocyclic lactone endectocides (moxidectin, ivermectin, and eprinomectin) was evaluated on cattle against *R. microplus* using two different treatment regimes. A single treatment regime with each endectocide showed that fewer ticks per calf were recovered from all treated calves than from untreated cattle, but the level of control among the three treatments was similar (range; 78.7–87.7%) against all stages of ticks on the calves at the time of treatment. The engorged female and egg mass weights of all treated ticks were less than that of untreated ticks. Among the treated groups, the ivermectin and eprinomectin-treated females weighed less and produced lower weight egg masses than those from moxidectin-treated cattle. In a double application treatment regime with a 4-d interval between treatments, there were fewer ticks per calf recovered from the treated cattle than from untreated cattle. In addition, all treated females weighed less and produced lower weight egg masses than those from untreated cattle. Control with moxidectin (90.3%) was lower than with either ivermectin (98.9%) or eprinomectin (99.7%). The mean female and egg mass weight of the ivermectin and eprinomectin-treated groups was also less than that of the moxidectin treatment. A single application treatment against either 18- or 20-d-old adult ticks indicated that both moxidectin and ivermectin were less effective against 20-d-old ticks that were nearer to completing their parasitic development on the animal. In contrast, eprinomectin was the only endectocide tested that was equally effective against both 18- and 20-d-old ticks.

Davey *et al.* (2001) evaluated the efficacy of ivermectin administered orally to cattle infested with all parasitic stages of *R. microplus* ticks. Ivermectin capsules were administered to two separate groups of cattle at a dose rate of either 25 or 50 mg/kg for a period of 21 consecutive days. A third group of calves received a placebo capsule each day and served as a control. Although the overall control achieved at both doses of ivermectin was >99% against all parasitic stages. The 50 mg/kg/d dose was significantly more effective than the 25 mg/kg/d dose against each developmental stage of the tick. Each ivermectin treatment dose significantly reduced female tick burden. However, the 50 mg/kg/d treatment was significantly more effective in reducing tick numbers, regardless of the developmental stage of the ticks. Both engorgement weight and egg mass weight of females were significantly lower in the ivermectin treated groups than were observed in the untreated group.

Bridi *et al.* (2001) conducted a study in cattle experimentally infested with *Psoroptes ovis* to compare the prophylactic effect provided by a long-acting injectable formulation of ivermectin with that of a commercially available injectable formulation of doramectin. Thirty Holstein steers were used. Animals were allocated by restricted randomization based on Day 0 b. w., forming six replicates of five animals each. Live mites were found in 33, 67 and 83% of the untreated controls. No *P. ovis* mites were found in steers treated with long-acting ivermectin. Those animals showed lower ($P<0.05$) mite counts than untreated controls at 21 and 28 days after challenge. These results indicate that the long-acting injectable ivermectin formulation prevents *P. ovis* infestations for at least 56 days after treatment. Doramectin injectable formulation, used at 200mcg/kg, did not have a prophylactic effect at 35 days after treatment.

Hannan *et al.* (2001) performed a study to know the efficacy of Ivomec® pour-on against gastrointestinal nematodes, lice and ticks in goats. For this purpose 30 hoseholds in Boyra village of Kotowali upazila under Mymensingh district, each rearing at least one goat were included. After initial screening 30 goats infected with both endo and ecto parasites were selected amongst the 15 goats were

brought under the trial for evaluation of efficacy of ivermectin. Ivermecti
applied on the vertebral column of each animal at the dose rate 500 µg/kg
They found that ivermectin was 100% effective in all treated goats from da
The goats were remained free to ectoparasites upto 28 days post treatment wh
the experiment was terminated.



Chapter 3

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

The experiment was conducted in the department of Physiology and Pharmacology of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. The experiment was carried out for 28 days from August to September, 2013.

3.1 Collection of animals

Fifteen calves of both sexes aged between 5 to 6 months were primarily selected in this study. The calves were collected from the villages near HSTU campus, Dinajpur, namely Subra and Karnai. All the calves were examined for the presence of ticks, and the efficacy of neem leaves extract and ivermectin were evaluated against these naturally infested ticks.

3.2 Collection of plant material

Neem leaves were selected for its effectiveness against tick infestation in calves. Mature and disease free neem leaves were collected from HSTU campus.

3.3 Collection of drugs

Injectable ivermectin preparation was purchased from local market, Dinajpur.

3.4 Neem leaves extract preparation

The leaves were dried and powdered. For the preparation of 15% neem leaves extract, 15 g of leaf powder was mixed with 100 ml distilled water and stirred for 30 minutes at 6000 rpm. It was kept overnight at 4°C and the supernatant was collected. This was used as the crude leaf extract to study.

3.5 Experimental design

Calves, naturally infested with ticks, were randomly divided into 3 equal groups namely A, B and C for assessing the efficacy of neem leaves extract and ivermectin against tick infestation.

Group A: was kept as infected control without giving any treatment.

Group B: was treated with 15% neem leaves extract (spray)

Group C: was treated with ivermectin @ 200 µg/kg body weight S/C.

All the calves of treated and control groups were closely observed for 28 days after treatment and following parameters were studied

a) Clinical parameter (severity of infestation, b. w., condition of hair coat and feeding efficiency).
b) Hematological parameters (Hb, PCV, ESR and TEC)

During experimental period, both control and treated calves were kept in animal shade. Wheat bran, rice polish, maize and salt are mixed together and supplied to the calves up to 1-2 kg approximately daily. All the calves were allowed for free pasture grazing for 2-3 hours daily. Adequate amount of water was also supplied.

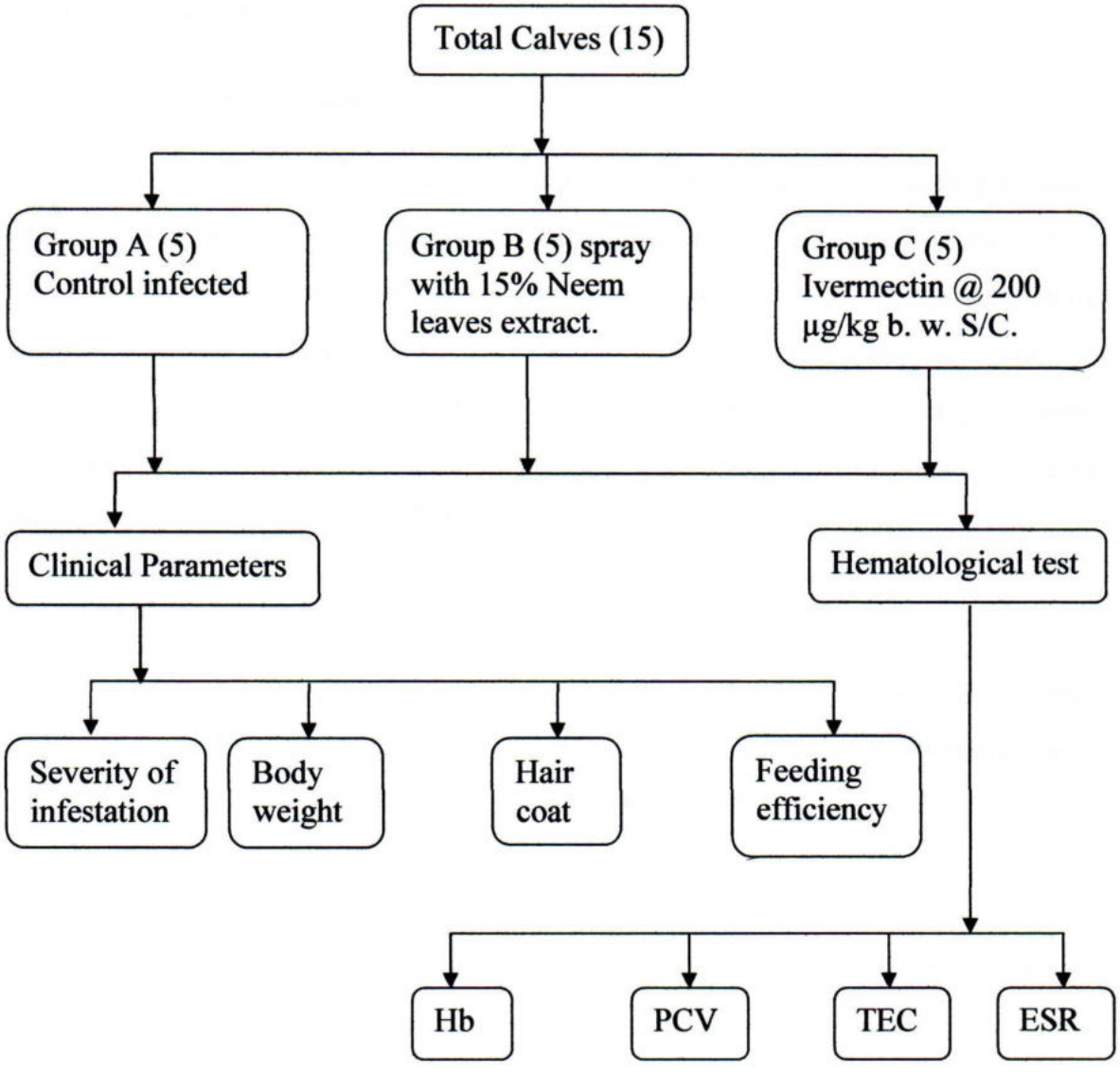


Fig. 2: Schematic diagram showing experimental design

3.5.2 Hematological parameters:

Blood samples were collected from Jugular vein of calves of both control and treated groups in vials containing anticoagulant (Sodium Citrate 3.8%) at day 0, 7^{th}, 14^{th}, 21^{st} and 28^{th} day of treatment to determine the effect of neem leaves extract and ivermectin on the hematological parameters such as TEC, Hb, PCV and ESR. These hematological parameters were determined following the procedures as described previously (Coffin, 1955).

3.6 Statistical analysis

Data obtained from the experiment on body weight, hematological parameters such as (Hb, PCV, TEC, ESR) were analyzed statistically between control and treated groups by using students't' test following the standard methods by Zar (2002).

Fig. 3: Neem Tree (*Azadirachta indica*)

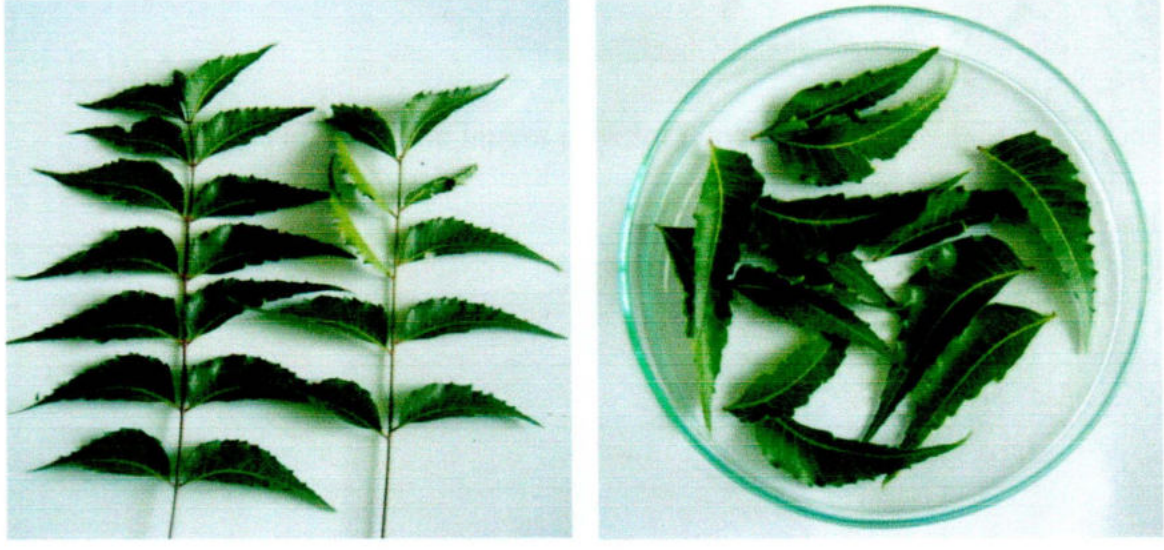


Fig 4: Leaves of Neem *(A. indica)*

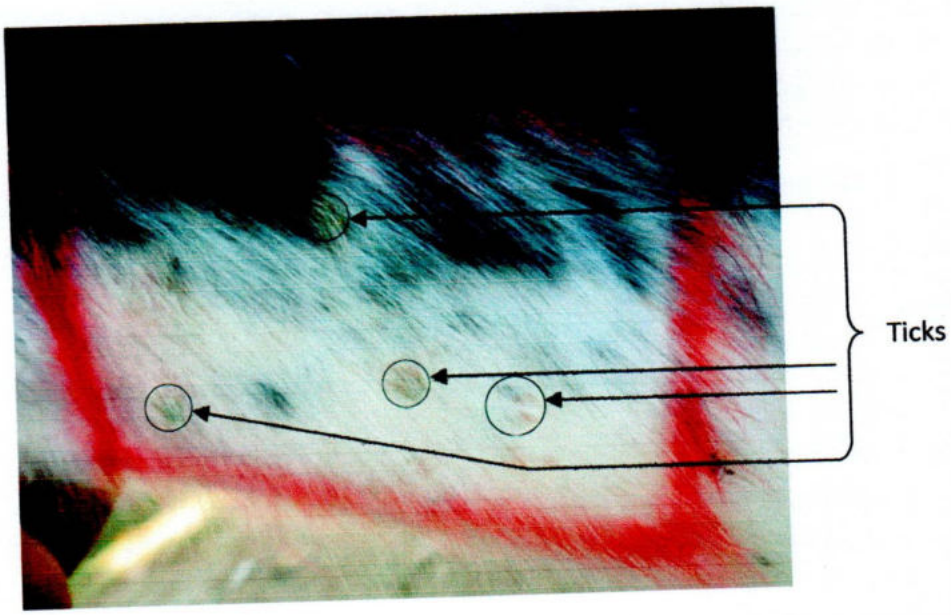


Fig. 8: Selected area in the dewlap region showing tick infestation and level of tick burden in a calf before treatment

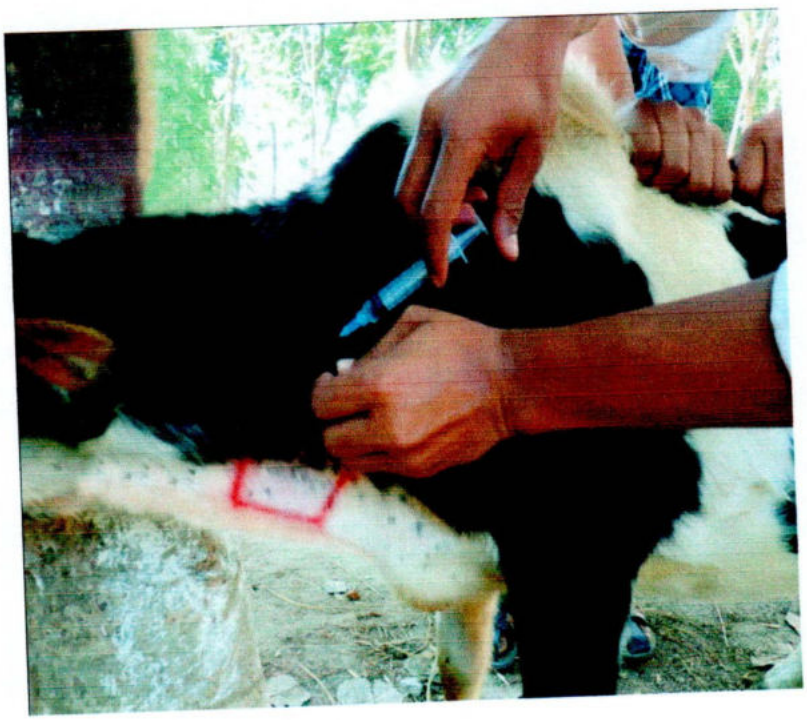


Fig. 9: Subcutaneous injection of ivermectin in a calf

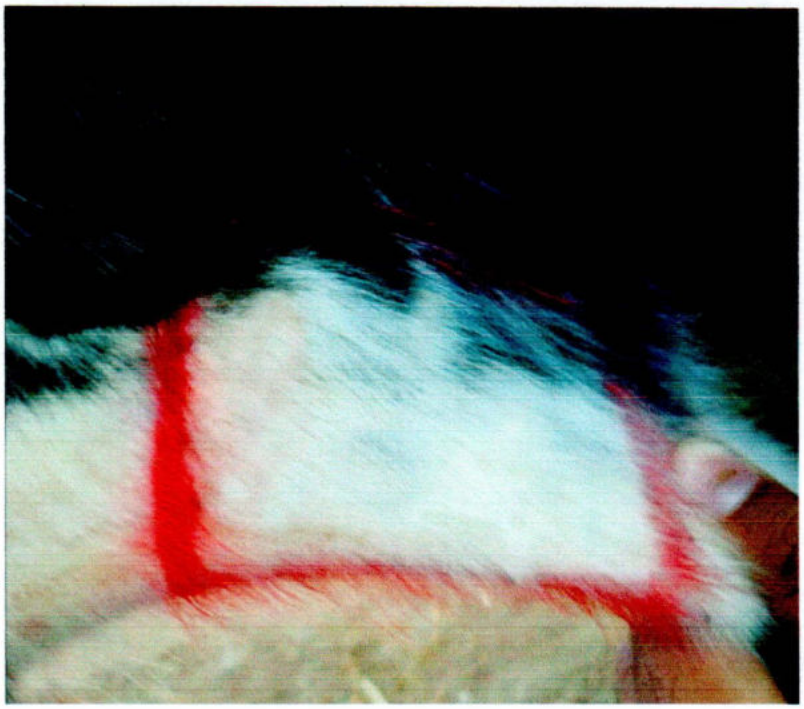
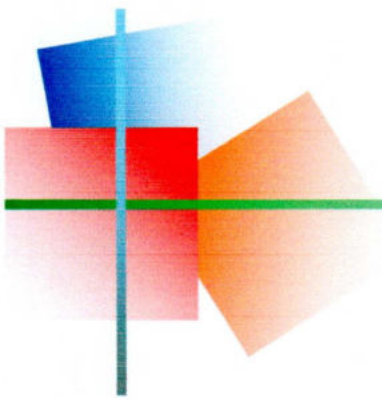


Fig. 10: Tick counting area after treatment with ivermectin in a calf (animal was treated with ivermectin @ 200 µg/kg b. w. and at the 7th day post treatment the selected area was completely free from tick)



Chapter 4

RESULTS

CHAPTER 4

RESULTS

A research work was conducted to evaluate the effect of neem leaves extract (spray) and ivermectin (injectable formulation) on following parameters.

I. The efficacy of neem leaves extract (spray) and ivermectin against tick infestation in calves.
II. The effect of neem leaves extract (spray) and ivermectin (injectable) on body weight in calves.
III. The effect of neem leaves extract (spray) and ivermectin (injectable) hematological parameters (TEC, Hb, PCV and ESR) in calves.

4.1 Neem leaves extract and ivermectin significantly reduced tick burden in calves

In the group B neem leaves extract at 15% spray was found to be 68.8% effective against tick infestation. On the day '0' the number of mean value of ticks was 12.20±0.60 in the selected area but on the day '28' the number of mean value of ticks was 3.80±0.46. However, ticks gradually 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 'A', the number of ticks increased gradually on 7^{th}, 14^{th}, 21^{st} and 28^{th} day of treatment. On the day '0' the number of mean value of ticks was 12.80±0.77 in the selected area but on the day '28' the number of mean value of ticks was 15.60±0.37. On the other hand, on group C calves treated with ivermectin at the dose rate of 200 µg/kg b. w. showed 100% efficacy i.e. no ticks were found by physical examination on the 7^{th}, 14^{th}, 21^{st} and 28^{th} day of post treatment.

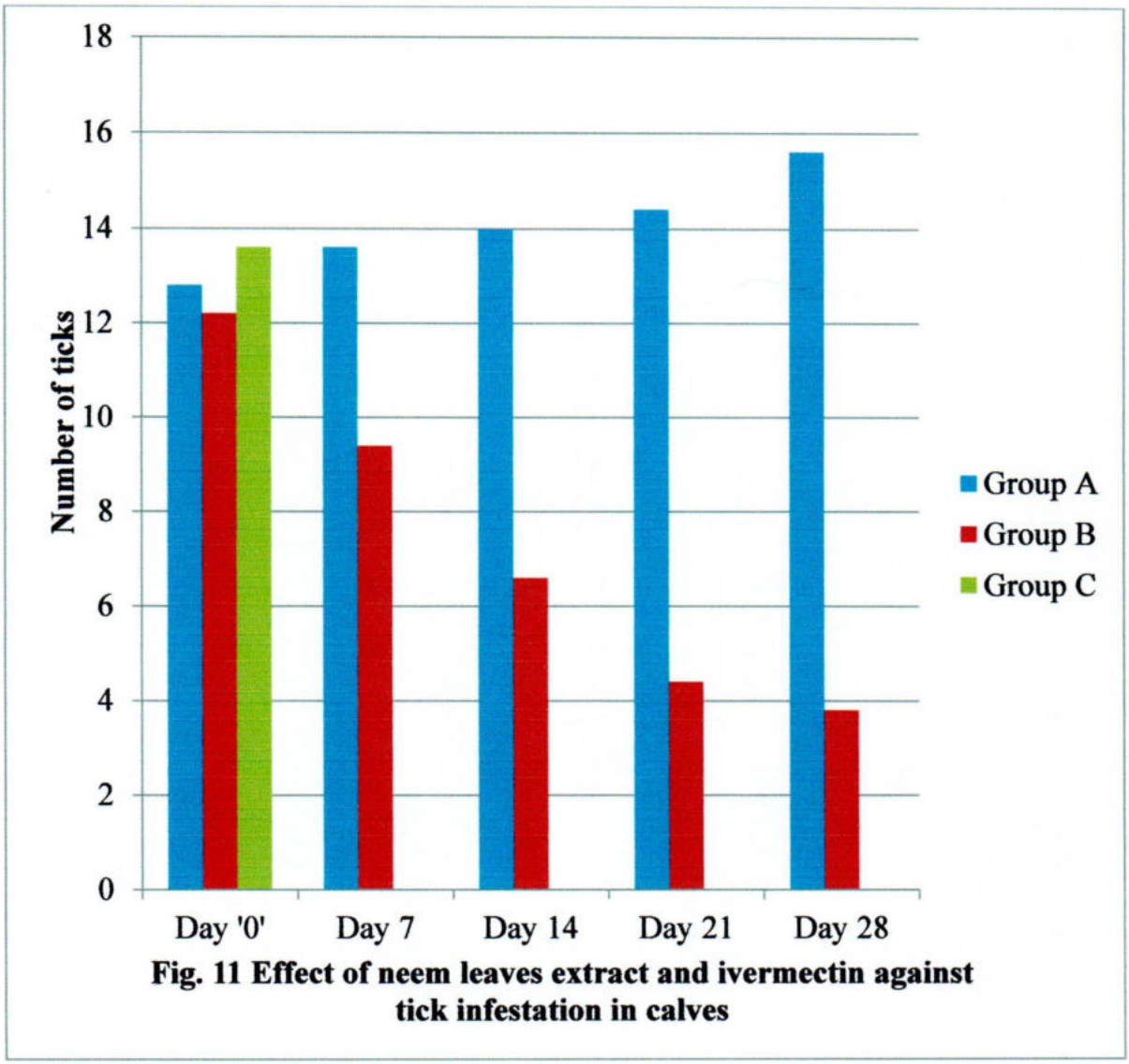


Fig. 11 Effect of neem leaves extract and ivermectin against tick infestation in calves

4.1.1 Neem and ivermectin improves hair coat

On day '0' (pre-treatment) the hair coat of all infected calves was rough with discolored hair. In group B and C, after treatment with neem leaves extract and ivermectin, the hair coat started to become smooth and shiny gradually and on 28th day of treatment the hair coat of the treated calves was almost alright. On the other hand, the hair coat of the infected control group A became more rough and discolored.

4.1.2 Treatment improves feeding efficiency

Feeding efficiency increased following neem leaves extract and ivermectin treatment. On the other hand, in the infected control group A, the feeding efficiency gradually decreased. In the neem leaves extract and ivermectin (injectable) treated calves (group B and C) feed intake increased gradually upto 1-2 kg approximately in comparison to pre-treatment ('0' day) period. However, in the control group A the feed intake decreased gradually during the post-treatment (7th, 14th, 21st and 28th) period.

4.1.3 Neem and ivermectin increases body weight

On the day '0' the mean initial b. w. of group B and C, treated with neem leaves extract (spray) and ivermectin (injectable) were 22.488 kg and 20.01 kg, respectively. However, on the 28th day of post treatment the mean values of body weight were 24.80kg and 24.22 kg, respectively indicating b. w. of calves increased significantly in treated groups. In control group (A), body weight of calves decreased upto 6.63% on 28th day of treatment. On the other hand, the b. w. increased in groups (B and C) to the extent of 3.11%-4.26%.

Table 2. Effects of neem leaves extract and ivermectin on body weight (kg) in calves

Groups	Treatment	Pre-treatment	Post-treatment			
		'0' day	7th day	14th day	21st day	28th day
A	Control (infected)	22.39±0.167	22.24±0.162 (0.80)	21.52 ±0.193 (4.12)	21.24± 0.103 (1.61)	20.17±0.173 (6.63)b
B	Spray with 15% neem leaves extract	22.49±0.087	23.41±0.144 (4.77)	23.63±0.115** (1.11)	23.72±0.201** (0.55)	25.80±0.80** (4.26)a
C	Ivermectin @ 200 µg/kg b. w. S/C	20.01±0.28	22.52±0.113 (0.54)	23.18±0.110** (3.46)	23.59 ±0.165* (2.07)	24.21±0.082 (3.11)a

Values given above are mean±SE of 5 calves

* Significantly increased (P<0.05)

** Significantly increased (P<0.01)

(%a) = Percent of increased a

(%b) = Percent of decreased b.

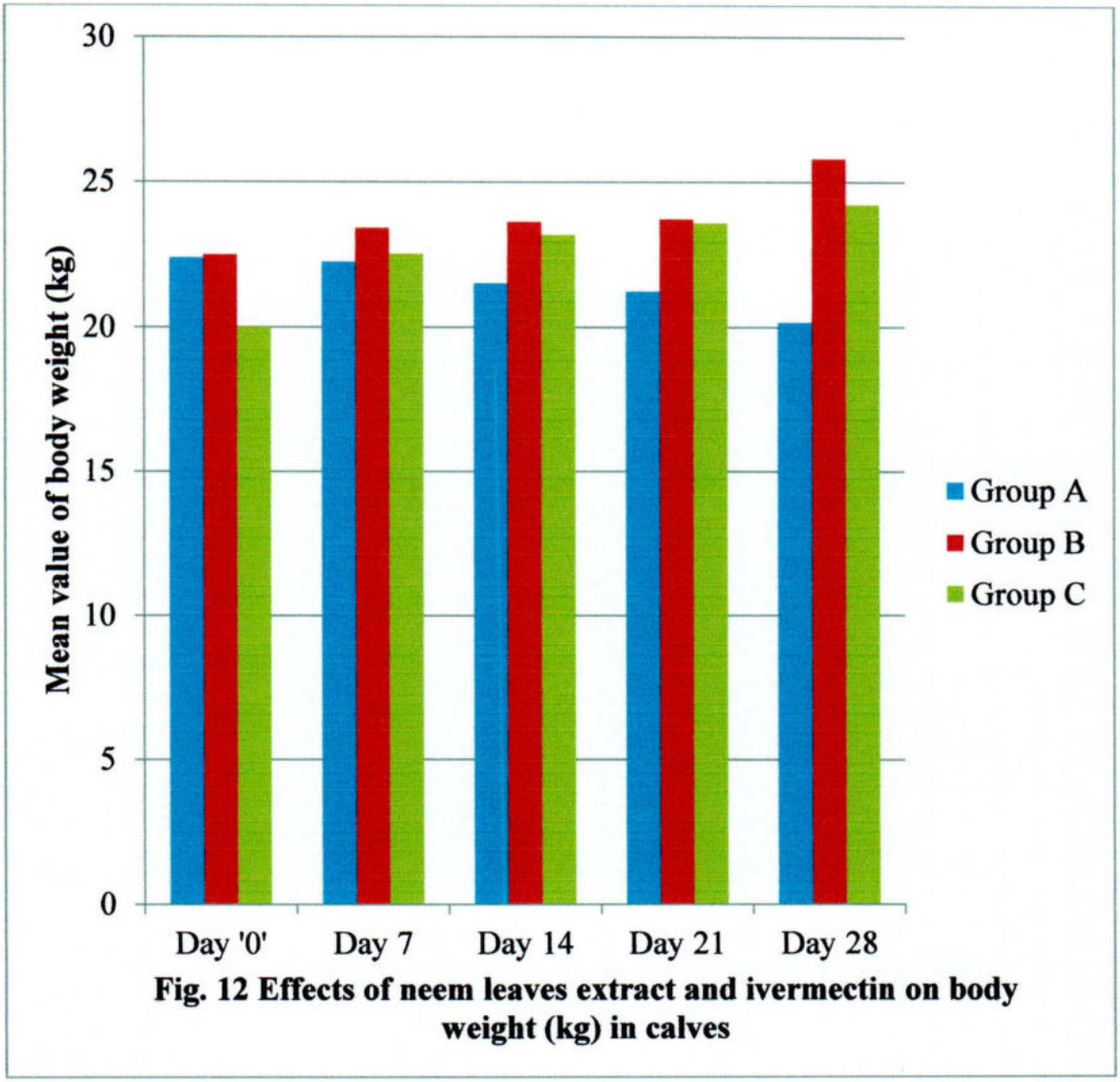


Fig. 12 Effects of neem leaves extract and ivermectin on body weight (kg) in calves

4.2 Treatment improves hematological parameters

a) Hb content

In control group (A), Hb content decreased upto 4.52% on 28th day of treatment. On the other hand, the Hb contents increased in treated groups (B and C) to the extent of 1.10%-3.02%, respectively, indicating treatment significantly increased Hb content.

Table 3. Effects of neem leaves extract and ivermectin on Hb content (gm %) in calves

Groups	Treatment	Pre-treatment	Post-treatment			
		'0' day	7th day	14th day	21st day	28th day
A	Control (infected)	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)b
B	Spray with 15% neem leaves extract	7.14±0.117	7.38±0.116** (3.25)	7.66±0.108** (3.66)	8.60± 0.145** (10.97)	8.70±0.114** (1.10)a
C	Ivermectin @ 200 µg/kg b. w. S/C	6.46±0.144	7.32±0.097** (11.75)	7.56±0.150** (3.17)	8.34± 0.129* (9.35)	8.60 ± 0.195* (3.02)a

Values given above are mean±SE of 5 calves

* Significantly increased (P<0.05)

** Significantly increased (P<0.01)

(%a) = Percent of increased a

(%b) = Percent of decreased b.

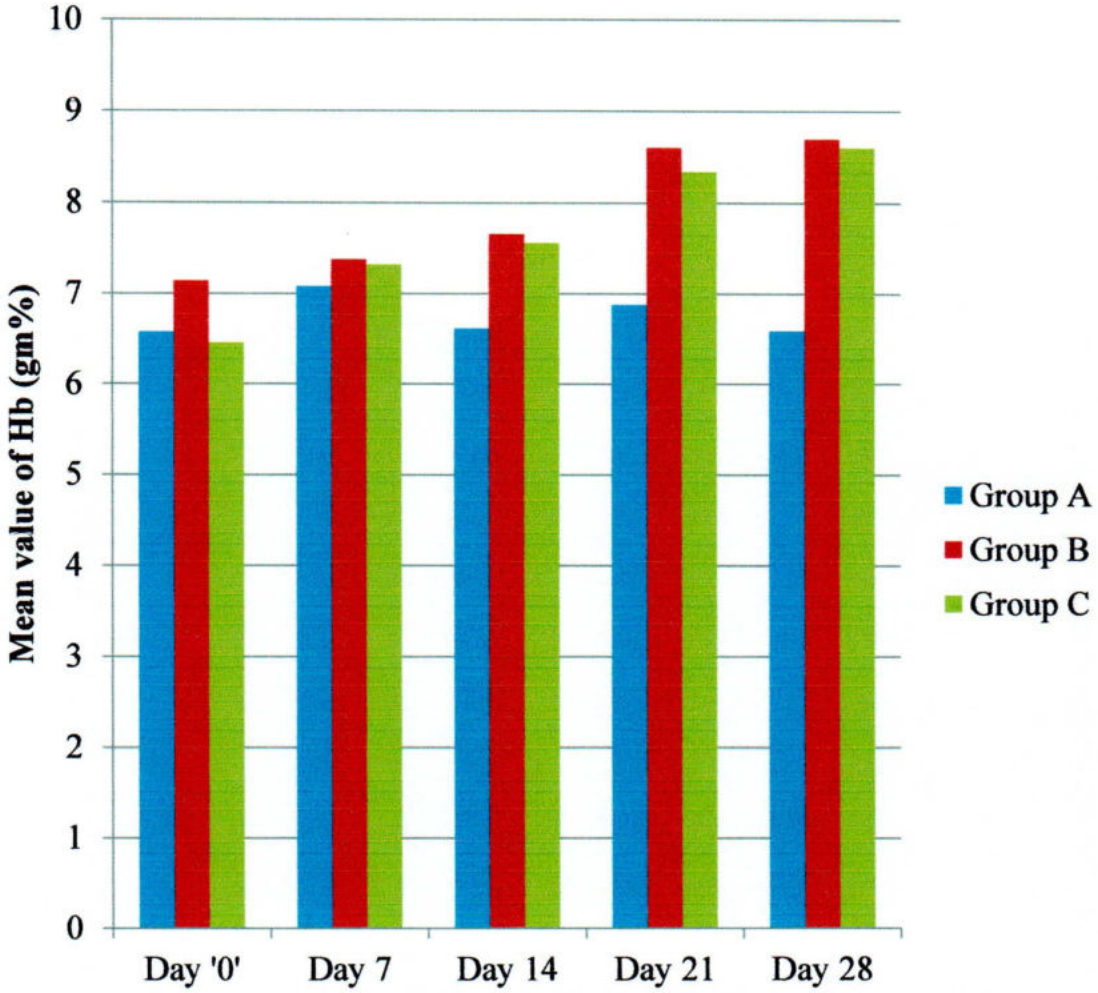


Fig. 13 Effects of neem leaves extract and ivermectin on Hb content in calves

b) Packed Cell Volume (PCV %)

In control group (A), PCV (%) values decreased upto 14.07% on 28th day of treatment. On the other hand, the PCV (%) values increased in all treated groups (B and C) to the extent of 1.11%-1.45%.

Table 4. Effects of neem leaves extract and ivermectin on Packed cell volume (PCV) (%) in calves

Groups	Treatment	Pre-treatment	Post-treatment			
		'0' day	7th day	14th day	21st day	28th day
A	Control (infected)	28.99±0.909	29.63±0.259 (2.17)	29.20±0.251 (1.49)	26.44±0.267 (10.44)	23.18±0.295 (14.07) b
B	Spray with 15% neem leaves extract	28.41±0.349	28.50±0.318 (0.34)	28.89±0.173** (1.33)	29.82±0.887 (3.11)a	29.39±0.381 (1.45)
C	Ivermectin @ 200 µg/kg b. w. S/C	29.75±0.307	29.58±0.229 (0.57)	29.49±0.159** (0.31)	30.69±0.433** (3.93)a	30.36±0.469** (1.11)

Values given above are mean±SE of 5 calves

** Significantly increased (P<0.01)

(%a) = Percent of increased a

(%b) = Percent of decreased b.

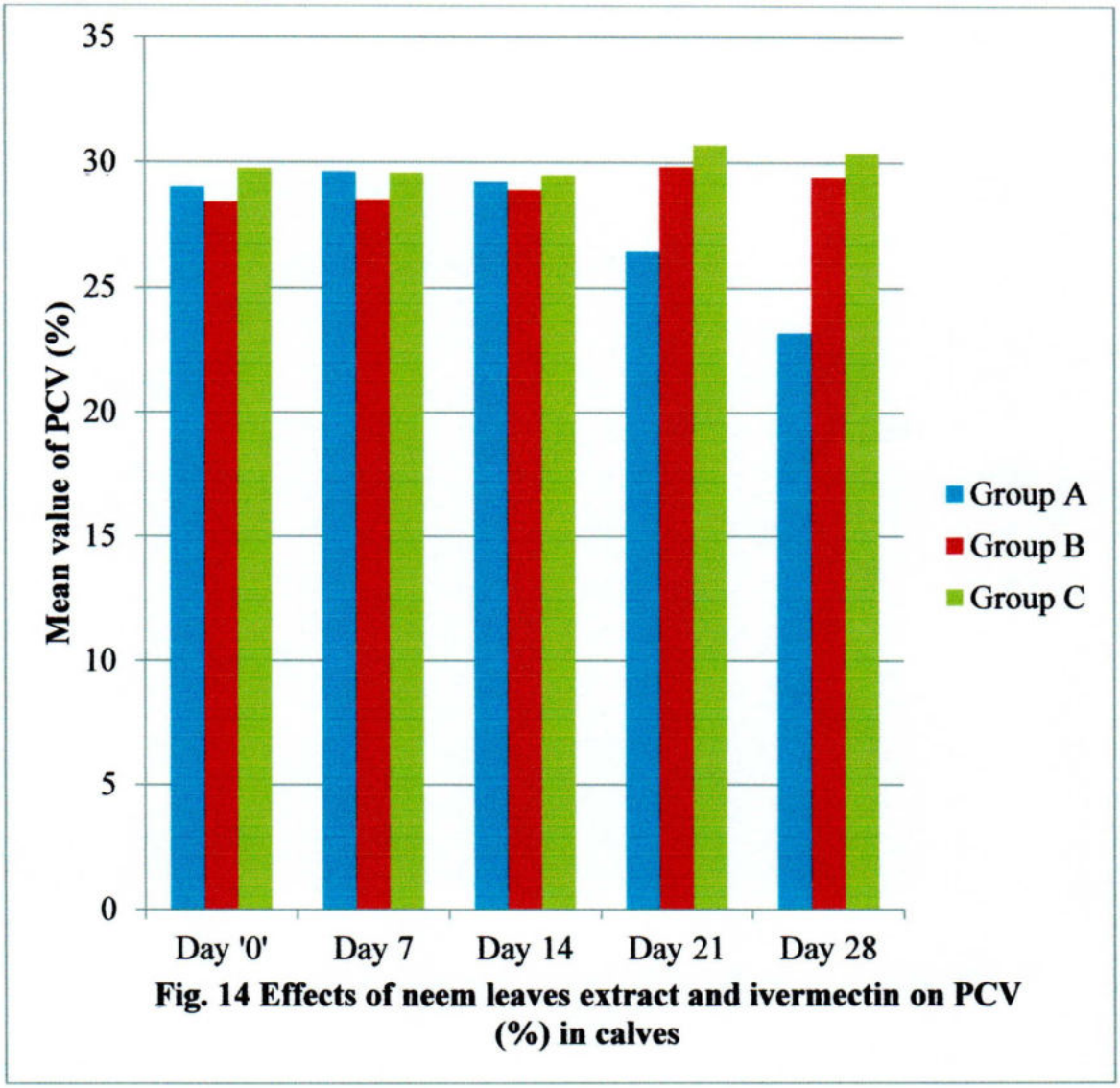


Fig. 14 Effects of neem leaves extract and ivermectin on PCV (%) in calves

c) Total Erythrocyte Count (TEC million/cu.mm of blood)

In control group (A), TEC values decreased upto 2.45% on 28th day of treatment. On the other hand, the TEC values increased in neem and ivermectin treated groups (B and C) to the extent of 5.20%-9.25% indicating treatment significantly increased TEC count in calves.

Table 5. Effects of neem leaves extract and ivermectin on TEC (million / cu. mm) in calves

Groups	Treatment	Pre-treatment	Post-treatment			
		'0' day	7th day	14th day	21st day	28th day
A	Control (infected)	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)b
B	spray with 15% Neem leaves extract	6.47±0.156	7.476±0.074 (13.46)	8.91±0.213** (16.09)	10.07±0.078 (11.50)	10.62±0.186** (5.20)a
C	Ivermectin @ 200 µg/kg b. wt. S/C	6.80±0.084	8.32±0.128** (18.27)	9.05±0.072** (8.03)	10.36±0.162* (12.72)	11.42±0.169* (9.25)a

Values given above are mean±SE of 5 calves

* Significantly increased (P<0.05)

** Significantly increased (P<0.01)

(%a) = Percent of increased a

(%b) = Percent of decreased b.

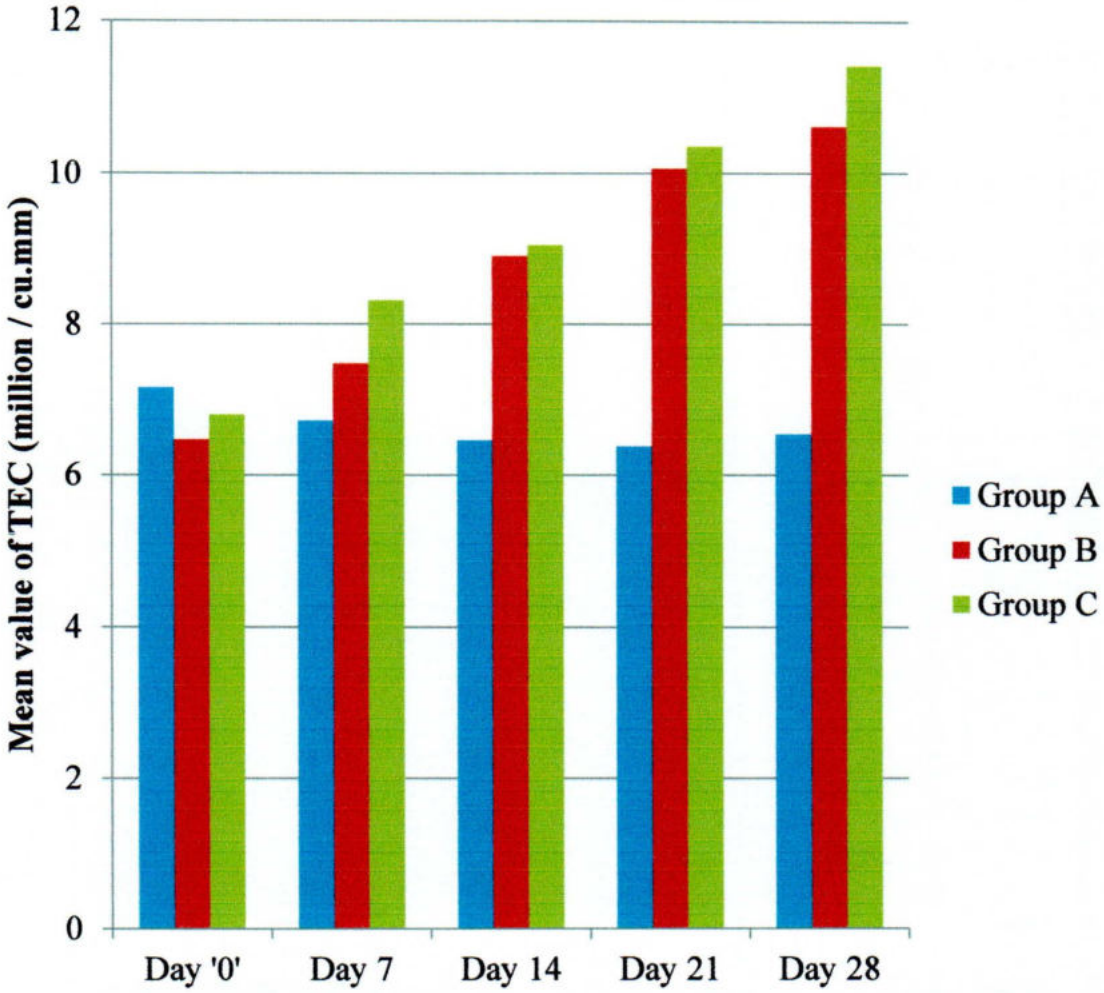


Fig. 15 Effects of neem leaves extract and ivermectin on TEC (million / cu. mm) in calves

d) Erythrocyte sedimentation rate (ESR mm/1st hr)

In control group (A), ESR (mm/1st hr) values increased upto 5.19% on 28th day of treatment. On the other hand, the ESR (mm/1st hr) values decreased in all treated groups (B and C) to the extent of 38.89% -176.67%.

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

Groups	Treatment	Pre-treatment	Post-treatment			
		'0' day	7th day	14th day	21st day	28th day
A	Control (infected)	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)a
B	spray with 15% Neem leaves extract	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)b
C	Ivermectin @ 200 µg/kg b. wt. S/C	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** (176.67)b

Values given above are mean±SE of 5 calves

* Significantly decreased (P<0.05)

** Significantly decreased (P<0.01)

(%a) = Percent of increased a

(%b) = Percent of decreased b.

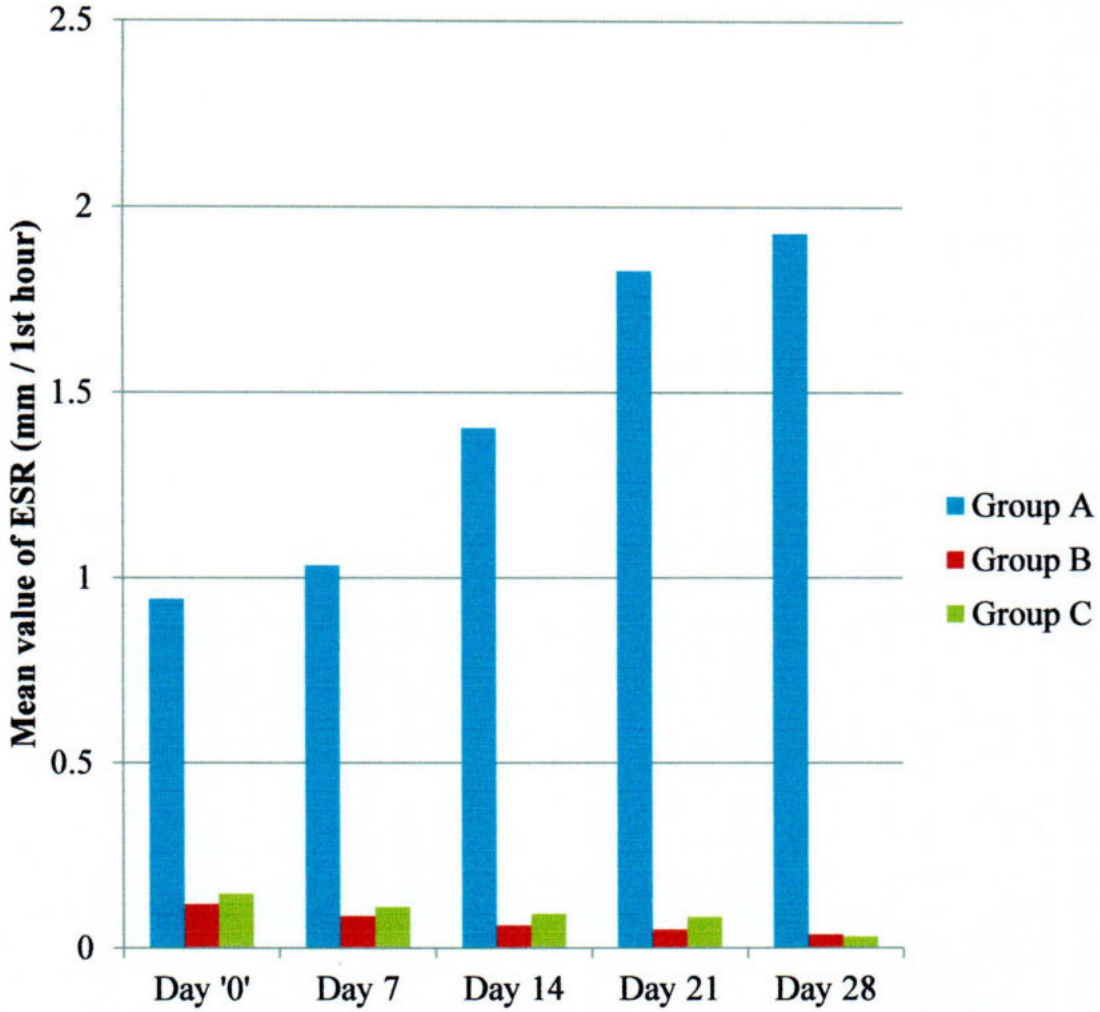
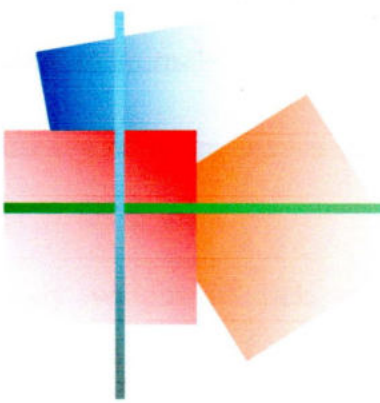


Fig. 16 Effects of neem leaves extract and ivermectin on ESR (mm / 1st hour) in calves



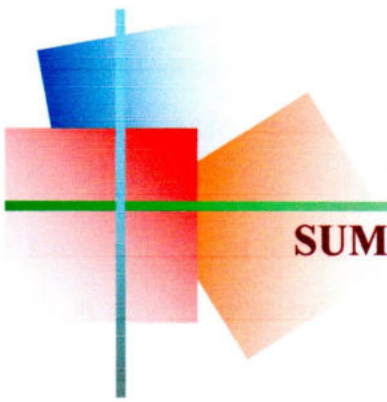
Chapter 5

DISCUSSION

The feeding efficiency was increased during the post treatment period in the following indigenous medicinal plant (neem spray) and ivermectin treated groups. It is well known that, due to tick infestation, the feeding efficiency is decreased. In the present study, the feeding efficiency increased due to better treatment showed by the indigenous medicinal plant (neem spray) and ivermectin. Also, the hair coats of all calves treated with indigenous medicinal plant (neem spray) and ivermectin were found smooth and shiny at the end of treatment.

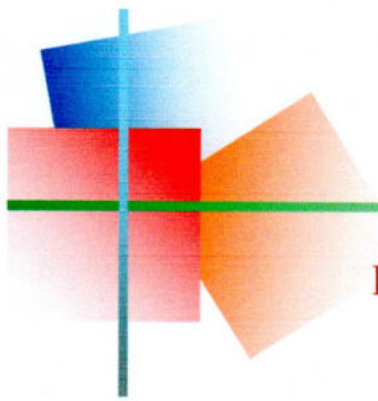
5.2 Efficacy of neem leaves extract and ivermectin on hematological parameters in calves

The hematological changes in calves affected with ticks were determined at pre and post treatment period dosed with neem and ivermectin. The mean value of Hb decreased in non-treated calves (control group). The results are in agreement with the reports of Nettelton and Beekett (1976) and Anosa (1977). In the present study, significant changes in Hb and PCV% were observed in the treated group of calves and this might be due to expulsion of blood sucking parasites from the body. The significant reduction in the sedimentation rate of erythrocytes in successfully treated group was observed and this may be due to recovery from inflammation induced by ectoparasites. There are very limited literatures showing the effects of neem and ivermectin on hematological parameters. However, Anil-Kumar and Joshi (1992), found increase of Hb level in ivermectin treated calves.



Chapter 6

SUMMARY AND CONCLUSION



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