Assessment of Adverse Effects of Ivermectin in Goats

THESIS

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In the partial fulfilment of the requirement

FOR THE DEGREE OF

Master of Veterinary Science

IN

(VETERINARY PHARMACOLOGY AND TOXICOLOGY)

By

Archana

Reg No. - M/VPT/57/2002-2003

DEPARTMENT OF VETERINARY PHARMACOLOGY AND TOXICOLOGY

BIHAR VETERINARY COLLEGE

PATNA - 800 014

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Dedicated to

MY ADORABLE

Parents

&

Parents in-laws

DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY
Bihar Veterinary College, Patna-800014
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CERTIFICATE - I

This is to certify that the thesis entitled **"ASSESSMENT OF ADVERSE EFFECTS OF IVERMECTIN IN GOATS"** submitted in partial fulfillment of the requirement for the degree of **"Master of Veterinary Science (Veterinary Pharmacology & Toxicology)"** of the faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar, is the record of bonafide research carried out by **DR. ARCHANA,** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that such help or information received during the course of this investigation and preparation of the thesis have been duly acknowledged.

12/4/05

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CERTIFICATE - II

We, the undersigned, members of the Advisory Committee of

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"ASSESSMENT OF ADVERSE EFFECTS OF IVERMECTIN IN

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(Archana)

Place : Patna

Date : 12 – 04 – 05

CONTENTS

❀❀❀❀❀

LIST OF TABLES

✸✸✸✸✸

LIST OF FIGURE

Figure No.	Description
9.	Showing effect of single s.c. injection of ivermectin at five time the therapeutic dose (3.3 mg/kg) at various time intervals in goats on serum cholestrol (mg/dl)
10.	Showing effect of single s.c. injection of ivermectin at five time the therapeutic dose (3.3 mg/kg) at various time intervals in goats on blood urea nitrogen (BUN in mg/dl)
11.	Showing effect of single s.c. injection of ivermectin at the five time of therapeutic dose (3.3 mg/kg) at various time intervals in goats on Serum Glutanate Pyruvate Transaminase (SGPT) (IU/L)
12.	Showing effect of single s.c. injection of ivermectin at five time the therapeutic dose (3.3 mg/kg) at various time therapeutic dose (0.33 mg/kg) at various times intervals in goats on serum glutamic oxaloacetate transaminase (SGOT) (IU/L)
13.	Showing effect of daily s.c. injection of ivermectin at the therapeutic dose (0.66 mg kg) for 14 days on various intervals of days in goats on total plasma protein (g/dl)
14.	Showing effect of daily s.c. injection of ivermectin at the therapeutic dose (0.66 mg/kg) for 14 days on various intervals of days in goats on blood glucose level (mg/dl)
15.	Showing effect of daily s.c. injection of ivermectin at the therapeutic dose (0.66 mg kg) for 14 days on various intervals of days in oats on serum cholesterol.

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Chapter - 1

Introduction

INTRODUCTION

Ivermectin, a broad spectrum antiparasitic drug with ecto and endo antiparasitic activity is currently widely used in many countries including India. It is registered in over 60 countries for use in cattle, sheep, horse, goat, swine, camel, reindeer, bison and dog (Di Netta, 1989). Ivermectin is active against two major phyla of animal parasites, the nemathelminthes and the arthropoda (Campbell *et al.*, 1983). Apart from other antiparasitic use, all gastro-intestinal nematodes, lung nematodes, certain ecto parasites of cattle, horse, sheep, swine and sarcoptic mange of dogs are treated; heart worms are also effectively eliminated.

Goat (*Capra hircus*), undoubtedly the first ruminant domesticated by human and it has been associated with man for more than 10,000 years. Goat, a versatile species of animal is reared mainly for meat and milk purpose apart from its valuable hides. Besides, pashmina goat contributes better wool to garment industries in India. Goat is popularly known as "poor man's cow" in India and as "wet nurse of infants" in Europe. Goat milk is cheap, wholesome, easily digestible and nutritious. Goat can play a significant role by enhancing the socio-economic status of marginal farmers and landless labourers. Hence, it becomes necessary to provide a better health

coverage to this valuable species by achieving the new dimension through the use of effective drugs in preventing and curing various disease including diseases caused by ecto and endo parasites. Whenever a drug is introduced in clinical practice, precautions should be taken for the possible side effects due to over dosage, long term use and species susceptibility etc. Reports of toxicity studies were available in mice (Campbell *et al.*, 1979), calves (Lancaster *et al.*, 1982), cattle (Lancaster *et al.*, 1991; Basu *et al.*, 1992; Rehbein *et al.*, 1997; Islam *et al.*, 1999), donkeys (Hayatee *et al.*, 1989) and Sheep (Anil Kumar *et al.*, 1992). But, few toxicity studies were conducted in goats. Although ivermectin holds a wide margin of safety but animals like horse, dog, cattle and swine are prone to its toxicity. Toxicity of ivermectin shows variable degree of clinical symptoms such as mydriasis, CNS depression, restlessness, ataxia, tremor, drooling of Saliva, recumbency and death. The most common cause of ivermectin toxicity is administration of excessive dosage (10-20 times the recommended dose). Breed sensitivity to lower dose occurs in some dogs as in the Collie or Australian shepherd. Although not every "Sensitive" animal in the breed is affected. There may be a genetic component in sensitivity to animals. Dogs, especially collies appear to be idiosyncratically sensitive to ivermectin. Clinical observations after oral administration of ivermectin were carried out by Paul *et al.* (1987).

Ivermectin-induced toxicities in collies were studied by Tranquilli *et al.* (1989). Ivermectin toxicosis in Australlian shepherds was studied by Hadrick *et al.* (1995). Literature on toxicity of ivermectin in goats are scare.

Keeping in view the above mentioned facts, the present investigation was carried out in goats with following specific objectives :-

(1)   Toxicity study of ivermectin following its therapeutic dose and at higher dose on single parentral administration.

(2)   Toxicity study of ivermectin following repeated parentral administration at therapeutic dose.

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Chapter - 2

Review

of

Literature

REVIEW OF LITERATURE

Ivermectin, a relatively new endectocide drug has been found to be promising. It is a macrolide endectocide, having excellent activity against a wide spectrum of gastro-intestinal nematodes, lung worms and heart worms as well as systemic activity against several ectoparasites of domestic animals at extremely low dosage. The excellent spectrum of activity against both nematodes and arthropods has resulted in embracing and coining the term "endectocide". It has practically no useful activity against trematodes, cestodes or protozoa.

HISTORY

Ivermectin, a macrolid endectocide produced from "Avermectin" which was initially isolated from soil organism *Streptomyces avermitilis*. The compounds were tested at the kitasato institute in Japan. It was tested in a variety of biological assays in Merck Laboratories. One of the cultures was found to be active against nematode parasites. It yielded a series of novel macrocyclic lactones, named avermectins, which proved to be active against a variety of nematodes and arthropods. An extensive programme of chemical modification resulted in the selection of ivermectin (22, 23-dihydro-avermectin B_1) for commercial development. It was shown that ivermectin is absorbed rapidly after oral or parentral dosing and

is excreted almost entirely in faeces. Ivermectin is lipid soluble and hydrophobic leading to more persistent therapeutic tissue level.

CHEMISTRY

Ivermectin occurs as an off white to yellowish powder. It is very poorly soluble in water (4 µg/ml), but is soluble in propylene glycol, polyethylene glycol and vegetable oils. Ivermectin is photo labile in solution. It is a semi-synthetic derivative of the avermectins family. There are glycosides with a disaccharide attached to the hydroxyl group at C_{13}. The two identical sugars have been identified as L-oleandrose, a dideoxy-methyl-aldohexose. The chemical structure of ivermectin is shown below :

Fig. : *Chemical Structure of Ivermectin*

MECHANISM OF ACTION

Ivermectin acts by stimulating release of the inhibitory neurotransmitter GABA. GABA binds to the GABA receptor-chloride

channel complex of post synaptic neuronal membrane causing an influx of chloride ions. Influx of chloride ions hyperpolarizes the neuronal membrane making them less excitatory and decreasing nerve transmission. The hyperpolarization of neuronal membrane mediates a paralysis followed by death of parasite. Ivermectin paralyses the nematodes and arthropods by disrupting GABA mediated transmission of nerve signals (Campbell, 1986).

TOXICITY STUDIES

Ivermectin, an endectocide is the most frequently used drugs, which is highly effective against arthropods and nematodes. Their dung residues enhance the risk of adverse effect on non-target invertebrates and on the general grazing ecosystem. In this scenario, our objective is to review current knowledge of the agro environmental and biodiversity impact and risks of endectocides including ivermectin. The most common cause of its toxicity is administration of excessive dose (10-20 times the recommended dose). Toxicity studies of ivermectin have been mainly conducted in dogs (especially Collies, Beagles) elephant, horse, sheep and cattle apart from laboratory animals. The toxicity studies noted in different species are given below :

1.   **Mice :**

A study was conducted to evaluate the potential toxicity of prophylactic antiparasitic treatment on strains of mice that are

commonly used as experimental models and in genetic engineering. Survey on members of strains and age of mice for toxic reactions during treatment regimens that of combined anthelmintic and antiacaricidal agents, three experimental protocols (ivermectin, piperezine and dichlorovos in combination, ivermectin alone and fenbendazole/pyremethrin or fenbendazole/dichlorovos) were evaluated. The data suggest a potential for toxicity regimens should be initiated with caution (Toth *et al.*, 2000).

The experiments were carried out with 170 white mice divided into 4 groups. The 1st group consisted of 72 intact animals, administered s/c Ivomek (ivermectin @ 0.01 ml/kg and oxfendazole @ 10 mg/kg) per os ; group 2 consisted of 15 animals with induced trichocephaliasis, administered no anthelmintics. The group 3 consisted of 72 animals were infected with Trichecephalus muris invasion eggs and administered anthelmintic agents on day 46 of post infection. The group 4 consisted of 11 intact animals neither infected nor treated (reference group). The animals were decapitated 3, 12, 24 and 72 hr after the drug administration and histological examination of the kidney was carried out. The findings evidence that the morphologic changes in the kidney of mice with trichocephliasis are characterized by alternating proliferative process. Both the drugs used were found characterized by a more or less pronounced nephrotoxicity. Ivomek induced the most marked structural changes

7

in the kidneys of intact animals. Changes in the kidneys of intact mice were manifested after the drug administration than in intact animals where no antihelmintics was administered (Chebyshev *et al.*, 1993).

2. Rat :

Avermectin C was evaluated for mutagenic activity in the Ames test with *Salmonella typhimurium* TA 97, TA 98 and TA 100. The dominant lethal assay on unibred albino rats at a dose of 2.25 mg/kg body weight (1/10 of the LD_{50}) and in the metaphase test on FIC BA × C57BI/6 mice in a dose of 4.5 mg/kg body weight (1/5 of the LD_{50}). The agent showed no mutagenic activity in any of the tests. The anaphase test on FICBA × C57BI/6 mice revealed no antimitotic activity of avermectin C.

3. Cats :

Acute toxic symptoms in cats will appear within 10 hour of ingestion. Symptoms may include agitation, vocalization, anorexia, mydriasis, rear limb paresis, tremors, and disorientation. Blindness, head pressing, wall-climbing, absence of oculomotor menace reflex and a slow & incomplete response to pupillary light may also be seen. Neurological symptoms usually diminish over several days and most animals completely recover within 2-4 weeks.

4. Dogs :

Two groups of collies were presented to the University of California, VMTH with ivermectin toxicity. Group I comprised of 5 adults collies that received at least 500 mg/kg ivermectin and

presented to the VMTH 3 hours after intoxication. Three dogs required mechanical ventilation and euthonised for financial reason; the remaining 2 dogs were comatosed but recovered in 5-7 days. Group II was comprised of 12 adult collies. After s.c. injection of 200-250 μg/kg ivermectin, these animals showed greater variation in severity of illness among individual; 5 animals progressed to stupor or coma, whereas 4 animals remained ambulatory. In most of these dogs clinical sign deteriorated from the day of intoxication until approximately day 6, from which time they showed gradual but steady improvement (Hooper et al., 2002).

In dogs, symptoms of acute toxicity rarely occur at single dosage of 2 mg/kg or less. At 2.5 mg/kg mydriasis occurs and at 5 mg/kg tremors occur. At doses of 10 mg/kg, severe tremor and ataxia are seen. Death occurred when dosage exceeded 40 mg/kg but the LD_{50} is 80 mg/kg. Dogs (Beagles) receiving 0.5 mg/kg p.os for 14 weeks developed no signs of toxicity, but at 1-2 mg/kg for the same time period, developed mydriasis and had some weight decreases. Half of the dogs receiving 2 mg/kg/day for 14 weeks developed symptoms of depression, tremors, alexia, anorexia and dehydration.

The Collie breed appears to be more sensitive to the toxic effects of ivermectin then other canine breeds This may be due to a more permeable blood brain barrier to the drug or drug accumulation

in the CNS of this breed. At the dosage recommended for heart worm prophylaxis, it is generally believed that the drug is safe to use in collies. Dogs who receive an over dosage of ivermectin or develop signs of toxicity (CNS, GI & cardiovascular) should receive supportive and symptomatic therapy (Paul *et al.*, 1989).

Wijnands-Kleukers *et al.* (1996) stated that the national poisons control centre is regularly conducted about ivermectin intoxication in dogs and that the use of ivermectin in comparison animals, and specially collies and bobtails is not without risk. For this reason, the off-label use of ivermectin for these dogs is not advised. There is currently a wide choice of selective drugs for the treatment of canine parasitic diseases.

5. Tortoise :

Five red footed tortoise (*Geochelone carbonaria*) treated for animal nematode parasitism with a single i.m. injection of ivermectin (0.4 mg/kg) were found in state of extreme paresis or flaccid paralysis. One tortoise recovered normal motor function over the next 7-10 days. The remaining tortoise died within three days of receiving ivermectin. The only consistent P.M. finding was a marked fatty changes in the liver; studies show that some paresis will occur with dosages as low as 0.05 mg/kg. At least three other species chelonians were found to be susceptible to ivermectin toxicosis at similar dosages of 0.1 mg/kg (Teare *et al.*, 1983).

6. Elephant :

Karesh *et al.* (1985) studied infestation by the elephant louce, *Haematomyzus elephantis* was diagnosed in 5 Asian elephants and 4 African elephants. The elephants were treated orally with an injectable ivermectin preparation. Dosage ranged from 0.059 mg/kg to 0.087 mg/kg. Within 48-72 hours post treatment, the lice became easier to be removed manually. 7 day post treatment, lice were not found. 5-6 week after the 1st treatment, ivermectin was given again using the same route and same dose. At the time of the 2nd treatment a few lice were present but no egg was found. Previously, ivermectin administered by i.m. route was found to be effective against lice; however, it caused local inflammation and soreness at the injection site and the route was discontinued.

7. Sheep :

Sheep were injected with upto 25 times the therapeutic dose of ivermectin. The only adverse effects were transient inappetence and swelling at the injection site with a return in 4 days. Following s.c. injection at 200 μg/kg dose repeated after 14 days, no important changes were observed in blood cell count or in protein or nitrogen metabolism, no adverse effects were observed on the blood ascorbic acid content and on glutathione, aminotransferase or leucine aminopeptidase activities (Remez *et al.*, 1989).

8.   Goat :

In a flocks of cashmere goat cited in Austrias (NW, Spain), diminished efficacy was observed following treatment with Netobimin and consequently a study to demonstrate the existence of anthelmintic resistance, and species of involved was conducted. Result from faecal egg count reduction test before and after treatment with Netobimin or ivermectin showed efficacy of 89.4% and 99.7% respectively. (Requezo-Fernandez *et al.*, 2002).

9.   Donkey :

Ivermectin was evaluated for its therapeutic efficacy against natural strongyles infection in donkeys. Eight donkeys treated with ivermectin by s.c. route at the dose rate of 200 µg/kg body weight. Blood samples from all the donkeys were collected on day 0, 7 and 21 for estimating haemoglobin (Hb in gm/dl), packed cell volume (PCV%), Red blood cell (RBC $X10^{12}$/l), white blood cells (WBC$X10^9$/l) and erythrocyte sedimentation (ESR in mm/hr) by the methods of Schalm *et al.* (1975). Serum samples separated from blood collected on day 0, 7 and 21 post treatment were analysed for total protein, aspartate aminotransferase, alanine aminotransferase. After investigation, ivermectin was found to be effective against natural strongylosis of donkey and the drug had no side effect.

Dipietra *et al.* (1982) have reported 99% efficacy of this drug in horses. Elevation in the activity of AST in infected animals may be associated with cell necrosis of tissue as reported by Cornelius (1980). None of the treated animals had shown any degree of toxicosis or local reaction at the site of injection with immediate or delayed which agree to the findings of Dipietra *et al.* (1984) and Asquith & Kulwich (1981). Following treatment, blood chemistry also did not suggest any toxicity in the donkeys. On the basis of faecal examination and haematological and biochemical changes it is concluded that ivermectin @ 200 µg kg is highly effective and safest drug for the control strongyles in donkey. (Hayatee *et al.*, 1989).

10. Cattle :

The toxicity of residues of ivermectin and maxidectin in cattle faeces collected at interval after treatment was carried out. 2 groups of 5 murray grey × averdean Angus steer were treated with injectable formulation of ivermectin and naxidectin, respectively. 3rd group was treated as control. Ivermectin treated steers produce dung that exhibit larval development of both *Musca vetustissima* and *Musca domestica* for 7-14 days after treatment (Wardhaugh *et al.*. 1996).

The efficacy of ivermectin pour on formulation (Ivomec pour on) against gastro-intestinal nematodiasis in cattle was studied. Its effect on blood parameters and the live weight gain of the host was investigated. The cattle harbouring the gastro-intestinal nematodes

were treated with ivermectin pour on formulation at the dose rate of 500 μg kg body weight. The faecal samples and blood parameters were investigated at day 0, 7, 14, 21 and 28 post treatment and live weight gain was evaluated at day 0 or 28 post treatment. The significant alteration of Hb, PCV and ESR of ivermectin treated cattle indicates the direct influence of ivermectin on blood parameters and the significant increase in haemoglobin content of blood confirms with earlier report (Anil Kumar *et al.*, 1992). The body weight gain in the ivermectin treated animals substantiated by the previous report (Purvis *et al.*, 1996 ; Rehibein *et al.*, 1997). The live weight gain in the ivermectin treated animals obviously lies in the fact that as the parasites are eliminated the feed are properly digested and the nutrients are absorbed and metabolized for the optimum use by the body. The absence of any adverse effect in host body indicates that ivermectin dose not hamper the normal physiology of the host (Islam *et al.*, 1999).

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Chapter – 3

Materials
and
Methods

MATERIALS AND METHODS

In the present study, five healthy female goats of local breed between 1-1½ years of age and weighing between 16 to 22 kg of body weight were used. The goats were maintained on dry fodder, greens and concentrate apart from 4-5 hrs of routine grazing. Water was given *ad lib*.

EXPERIMENTAL DESIGN

Ivermectin was administered separately in each of five healthy goats by subcutaneous (s.c.) route at therapeutic dose rate of 0.66 mg/kg once daily for acute toxicity study and blood samples were collected at 0, 4, 8, 12, 24 and 48 hours to know the adverse effects of the drug. After a washout period of 15 days, the drug was given at higher dose i.e. 5 times the therapeutic dose (3.3 mg/kg) s.c. once only and blood was collected at before (0 h) and at 4, 8, 12. 24 and 48 hrs. Again, after a washout period of 15 days, drug was given at the therapeutic dose (0.66 mg/kg) s.c. once daily for 14 consecutive days and blood samples were collected at 0, 1, 2, 4, 8 and 14 days to know the long-term effect of the drug.

DRUG USED

IVERMECTIN

Ivermectin (Neomec 1%) - an injectable commercial preparation containing in concentration of 10 mg/ml marketed by Intas Laboratories Limited, India was used in the present study.

COLLECTION OF BLOOD AND SERUM SAMPLES AND THEIR TIMINGS

Blood samples were collected at 0, 4, 8, 12, 24 and 48 h after s.c. administration of ivermectin in each of 5 healthy goats at the therapeutic dose rate of 0.66 mg/kg body weight once daily for acute toxicity. After a washout period of 15 days the blood samples were collected at 0, 4, 8, 12, 24 and 48 h after s.c administration of ivermectin in at the higher dose rate of 3.3 mg/kg body weight once only for acute toxicity study. Again after a washout period of 15 days the blood samples were again collected at 0,1, 2, 4, 8 and 14 days for study of long term effect of the drug after s.c. injection at therapeutic dose (0.66 mg/kg) once daily for 14 days.

A. Blood and Serum

Before collection of blood the sites around the jugular vein on either side of the neck of animals were aseptically prepared. Blood samples were collected in distilled water washed centrifuge tubes containing appropriate amount of suitable anticoagulant. For collection of serum, blood was collected in distilled water washed test tubes and placed in slanting position. Blood was then allowed to clot in test tubes at room temperature. The serum was then further centrifuged to collect clear serum. Blood was collected by vene-puncture with disposable 18G needles at various above noted time

intervals after drug administration. Blood and serum were transferred in different vials for different biochemical tests. The samples were then kept in refrigerator until various haematological and biochemical tests were carried out.

For total leucocyte count (TLC) and differential leucocyte count (DLC) blood was taken from ear vein.

ADMINISTRATION OF DRUG

(i) Toxicity study at the therapeutic dose (0.66 mg/kg)

Ivermectin (Neomec)injection containing 10 mg of drug per ml was injected at the dose rate of 0.66 mg/kg body weight by sub-cutaneous route once only in each goats.

(ii) Toxictiy at the higher dose i.e. 5 times the therapeutic dose (3.3 mg/kg)

Ivermectin (Neomec) injection containing 10 mg of drug per ml was injected at the dose rate of 3.3 mg/kg body weight by subcutaneous route once only in each goat.

(iii) Toxicity study at the therapeutic dose (0.66 mg/kg) for long term effect of drug

Ivermectin (Neomec) injection containing 10 mg of drug per ml was injected at dose rate of 0.66 mg/kg body weight once daily by s.c route in each goat for 14 consecutive days.

PARAMETERS FOR TOXICITY STUDIES

For toxicity analysis, the following parameters were estimated.

(A) CLINICAL PARAMETERS

   (i)   Toxicity signs and symptoms

   (ii)   Respiration rate/minutes

   (iii) Pulse rate/minute

   (iv)   Body temperature

   (v)   Ruminal movement/5 minute.

(B) HAEMATOLOGICAL PARAMETERS

   (i)   Haemoglobin (gm%)

   (ii)   Total leucocyte count (TLC)

   (iii) Differential leucocyte count (DLC)

(C) BIOCHEMICAL PARAMETERS

   (i)   Total plasma protein

   (ii)   Blood glucose

   (iii) Serum cholesterol

   (iv)   Blood urea nitrogen (BUN)

   (v)   Serum glutamate pyruvate transaminase (SGPT)

   (vi)   Serum glutamate oxalo transminase (SGOT)

A. CLINICAL PARAMETER

Respiration rate/min. pulse rate/min, Body temperature and ruminal movement/5 minutes were noted in various above noted time intervals in all the 5 healthy goats.

B. HAEMATOLOGICAL PARAMETERS

(i) Haemoglobin (Hb in gm/dl)

The haemoglobin content of blood samples were estimated by Acid Haematin method using Hellige and sahli's haemoglobinometer as per procedure described by Schalm et al. (1975).

(ii) Total Leucocyte Count (TLC)

The total leucocyte count of blood was done using improved Neubauer chamber by the method as described by Boddie and Goe (1962).

(iii) Differential Leucocyte Count (DLC)

The differential leucocyte count of blood was done by the method as advocated by Boddie and Goe (1962).

The details of all haematological methods are described in Appendix – I.

C. BIOCHEMICAL PARAMETERS

(i) Total plasma protein

The estimation of total plasma protein was done by Biuret and Bromocresol method as advocated by Reinhold (1953).

(ii) Blood glucose

The estimation of blood sugar was done by Folin and Wu method as described by Frakel et al. (1970).

(iii)   *Serum cholesterol*

The estimation of blood cholesterol was done by Ferric Chloride method as described by Wootton (1964).

(iv)   *Blood urea nitrogen (BUN)*

The blood urea nitrogen was estimated by Diacetyl Monoxime method as described by Wootton (1964).

(v)   *Serum glutamate pyruvate transaminase (SGPT)*

The estimation of SGPT was done by the 2,4- DNPH method as described by Reitman and Frankel (1957).

(vi)   *Serum glutamate oxalo transminase (SGOT)*

The estimation of SGOT was done by 2, 4-DNPH method as described by Reitman and Frankel (1957).

The details of all biochemical methods are described in Appendix II.

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Chapter - 4

Results

TOXICITY STUDIES OF IVERMECTIN

For conducting toxicity studies, apparently health goats were divided into three groups consisting of 5 animals in each group.

Group I - Ivermectin was injected at the therapeutic dose of 0.66 mg/kg s.c. as a single administration.

Group II - Ivermectin was injected at a dose of 3.3 mg/kg s.c. (5 times the therapeutic dose) as a single administration.

Group III - Ivermectin was injected at therapeutic dose (0.66 mg/kg s.c.) daily for 14 days.

The results of the study are noted below.

I. TOXICITY STUDIES AFTER A SINGLE THERAPEUTIC DOSE (0.66 mg/kg s.c.)

Toxicity study of ivermectin was conducted in each of 5 healthy goats after single s.c. administration of the drug at therapeutic dose rate of 0.66 mg/kg body weight. The blood samples were collected at 0 h (before administration of the drug) and at 4, 8, 12, 24 and 48 h post drug administration. The following parameters were studied and the results are noted below.

A. CLINICAL PARAMETERS :

(i) *Respiration rate :*

Effect of single s.c injection of 0.66 mg/kg body weight of ivermectin on respiration rate at different hours are presented in Table 1. The values of respiration rate/min at 0 h ranged from 22 – 24 with a mean of 23 ± 0.44. Analysis of data shows non significant difference between the value noted at 0 h (pre treatment) and 4, 8, 12, 24 and 48 h post drug administration. This clearly shows that the drug has no significant effect on respiration rate.

Table – 1

Effect of single s.c. injection of ivermectin (0.66 mg/kg) on various time intervals in goats on respiration rate/min.

Animal No.	TIME IN HOURS					
	0	4	8	12	24	48
1	22	24	23	25	22	22
2	24	23	24	22	23	21
3	23	24	23	22	21	21
4	24	23	22	21	20	20
5	22	24	23	25	22	22
Mean ± S.E.M	23.0 ± 0.44	23.6 ± 0.24	23.0 ± 0.31	23.0 ± 0.83	21.6 ± 0.50	21.2 ± 0.37

Table – 1A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S	M.S.	F
Between hours	5	22.17	4.43	3.664^{NS}
Error	24	29.2	1.21	

^{NS} = Non significant

(ii) Pulse Rate :

Table 2 presents the value of single s.c. injection of ivermectin (0.66 mg/kg) on pulse rate. Mean ± S.E.M value of 71.4 ± 0.56/min was noted at 0 h which slightly increased to 74.2 ± 1.24 and 79.0 ± 0.54 at 8 and 12 h, respectively. Thereafter, the value slightly decreased to 74.2 ± 1.24 and 74.2 ± 0.37 at 24 and 48 h, respectively. Analysis of data by single factor ANOVA (Table 2A) does not show any significant difference. Thus, it seems that ivermectin may not have any effect on pulse rate.

Table – 2

Effect of single s.c. injection of ivermectin (0.66 mg/kg) at various time intervals in goats on pulse rate/min.

Animal No.	Time in Hours					
	0	**4**	**8**	**12**	**24**	**48**
1	70	74	78	74	78	74
2	72	72	71	75	72	75
3	70	75	72	72	75	73
4	72	74	75	74	71	75
5	73	75	75	75	75	74
Mean ± S. E. M.	71.4 ± 0.56	74.0 ± 0.54	74.2 ± 1.24	79.0 ± 0.54	74.2 ± 1.24	74.2 ± 0.37

Table – 2A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Hours	5	31.7	6.21	1.78 [NS]
Error	24	83.6	3.48	

[NS] = Non significant.

(iii) Body temperature :

Table 3 presents the effect of single s.c. injection of ivermectin (0.66 mg/kg) on body temperature. Mean ± S. E. M. value of 102.52 ± 0.149 was noted at 0 h which increased non significantly to 102.72 ± 0.185, 102.88 ± 0.162, 103.2 ± 0.089, 103.24 ± 0.16 and 103.24 ± 0.16° F at 4, 8, 12, 24 and 48 h post administration of the drug.

Table – 3

Effect of single s.c. injection of ivermectin (0.66 mg/kg) at various time intervals in goats on body temperature (°F)

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	102.6	102.0	102.6	103.0	103.0	103.0
2	102.0	102.8	102.4	103.0	103.4	103.4
3	102.8	103.0	103.2	103.4	103.8	103.8
4	102.4	102.8	103.0	103.2	103.0	103.0
5	102.8	103.0	103.2	103.4	103.0	103.0
Mean ± S.E.M.	102.52 ± 0.149	102.72 ± 0.185	102.88 ± 0.162	103.20 ± 0.089	103.24 ± 0.160	103.24 ± 0.160

Table – 3 A

Showing analysis of data by single factor ANOVA.

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Hours	5	2.359	0.4718	3.978 [NS]
Error	24	2.848	0.1186	

[NS] = Non significant

(iv) *Ruminal movement :*

Effect of single s.c. injection of ivermectin (0.66 mg/kg) on ruminal movement is presented in Table 4. At 0 h the ruminal movement/5 min ranged from 4 to 7 with a mean 6.14 ± 0.50. Mean $\pm$ S. E. M. value of 6.20 ± 0.44, 6.20 ± 0.49, 6.22 ± 0.51, 6.24 ± 0.50 and $6.25 \pm 0.49/5$ min were obtained at 4, 8, 12, 24 and 48 h, respectively. These values do not differ significantly between hours (Table 4A), which denote that ivermectin may not have any significant effect on ruminal movement.

Table – 4

Effect of single s.c. injection of ivermectin (0.66 mg/kg) at various time interval in goats on ruminal movement (Rate/5min.)

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	5	6	8	7	5	6
2	4	6	5	4	6	5
3	7	8	5	6	7	6
4	6	7	7	8	6	7
5	6	5	6	7	8	7
Mean ± S. E. M.	6.14 ± 0.50	6.20 ± 0.49	6.20 ± 0.49	6.22 ± 0.51	6.24 ± 0.50	6.25 ± 0.49

Table – 4 A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Hours	5	2.4	0.48	1.23[NS]
Error	24	9.4	0.39	

[NS] = Non significant

B. HAEMATOLOGICAL PARAMETERS

(i)   Hemoglobin (Hb in g/dl) :

Various values of haemoglobin noted at different times (0, 4, 8, 12, 24 and 48 h) after single s.c. injection of ivermectin are presented in Table 5 and Fig. 1. The values of haemoglobin before drug administration (0 h) varied from 11 to 11.6 g/dl with a mean of 11.24 ± 0.11 g/dl. Mean ± S.E.M. values of 11.44 ± 0.07, 11.48 ± 0.10, 11.64 ± 0.07, 11.72 ± 0.08 and 11.8 ± 0.15 g/dl were noted at 4, 8, 12, 24 and 48 h, respectively, after s.c. administration of ivermectin. Analysis of data show non significant difference between hours (Table 5A) which indicates that ivermectin has no significant effect on haemoglobin.

Table – 5
Effect of single s.c. injection of ivermectin (0.66 mg/kg) at various times interval in goats on haemoglobin (Hb mg/dl)

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	11.0	11.2	11.2	11.4	11.6	11.2
2	11.0	11.4	11.4	11.6	11.6	12.0
3	11.6	11.6	11.8	11.8	12.0	12.0
4	11.2	11.4	11.4	11.6	11.6	11.8
5	11.4	11.6	11.6	11.8	11.8	12.0
Mean ± S.E.M.	11.24 ± 0.11	11.44 ± 0.07	11.48 ± 0.10	11.64 ± 0.07	11.72 ± 0.08	11.8 ± 0.15

Table – 5 A
Showing analysis of data by single factor ANOVA.

Source of variation	D.F.	C.S.S.	M.S.	F
Between Hours	5	1.0627	0.2125	3.877 [NS]
Error	24	1.3173	0.0548	

[NS] = Non significant.

(ii) Total leucocyte count (TLC) :

Effect of ivermectin on total leucocyte count (TLC) after single s.c. injection (0.66 mg/kg) is shown in Table 6. At 8 h, the mean ± S. E. M. of TLC was noted to be 9780 ± 60.41 mm^3 of blood, which non significantly increased to 9935 ± 68.73, 10005 ± 68.92, 10120 ± 56.12 and $10145 \pm 61.4.41$/mm^3 at 4, 8, 12 and 24 h post s.c injection, respectively. At 48 h, a non significantly decreased value of 10105 ± 42.13/mm^3 was noted as compared to 0 h. Analysis of data by single factor ANOVA reveals non-significant difference between hours (Table 6A). It seems that ivermectin has no significant effect on TLC.

Table – 6

Effect of single s.c. injection of ivermectin (0.66 mg/kg) at various time interval in goats on total leucocyte count (TLC)

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	10000	10175	10250	10300	10325	10025
2	9750	9900	10000	10050	10000	10050
3	9700	9750	9850	10000	10050	10050
4	9800	9900	10000	10050	10100	10150
5	9650	9950	10150	10200	10150	10250
Mean ± S.E.M.	9780 ± 60.41	9935 ± 68.73	10050 ± 68.92	10120 ± 56.12	10145 ± 61.44	10105 ± 42.13

29

Table – 6 A

Showing analysis of data by single factor ANOVA.

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Hours	5	492687	98537.4	0.54^{NS}
Error	24	4376500	182354.16	

^{NS} = Non significant.

(iii) Differential leucocyte count (DLC) :

Differential leucocyte count noted at different time (0, 4. 8, 12, 24 and 48 h) after single s.c. injection of ivermectin (0.66 mg/kg· is presented in Table 7. In parenthesis the original values concerted to arc sin values are presented for proper statistical analysis. Single factor ANOVA test reveals that there is non significant effect of ivermectin on different leucocytes viz., neutrophil, lymphocyte. moncocyte, eosinophil and basophil (Table 7A).

Table - 7

Effect single s.c. injection of ivermectin (0.66 mg/kg) at various times intervals in goats on DLC

Time in Hour

Animal Number	0 N	0 L	0 M	0 E	0 B	4 N	4 L	4 M	4 E	4 B	8 N	8 L	8 M	8 E	8 B	12 N	12 L	12 M	12 E	12 B	24 N	24 L	24 M	24 E	24 B	48 N	48 L	48 M	48 E	48 B
1	36% (36.87)	55% (47.87)	1% (5.47)	2% (8.13)	0% (0)	35% (36.27)	50% (45.00)	2% (8.13)	4% (11.54)	1% (5.47)	32% (34.45)	55% (47.87)	3% (9.00)	5% (12.02)	0% (0)	40% (39.23)	51% (45.57)	1% (5.47)	3% (9.98)	0% (0)	44% (41.55)	53% (41.23)	2% (8.13)	4% (11.54)	0% (0)	45% (42.13)	55% (42.13)	3% (9.08)	5% (12.02)	1% (5.41)
2	38% (38.06)	52% (46.15)	3% (9.98)	3% (9.98)	1% (5.47)	39% (38.05)	53% (46.23)	4% (11.54)	4% (11.54)	1% (5.47)	40% (39.23)	52% (46.15)	3% (9.98)	4% (11.54)	1% (5.47)	36% (36.87)	54% (47.20)	3% (9.98)	3% (9.98)	1% (5.47)	36% (36.87)	55% (47.82)	2% (8.13)	4% (11.54)	1% (5.47)	37% (37.47)	56% (48.45)	3% (9.98)	3% (9.98)	1% (5.47)
3	35% (36.27)	55% (47.87)	5% (9.98)	2% (8.13)	0% (0)	36% (36.87)	54% (47.29)	3% (9.98)	2% (8.13)	1% (5.47)	38% (38.06)	52% (46.15)	3% (9.98)	4% (11.54)	0% (0)	40% (34.23)	52% (46.15)	2% (8.13)	2% (8.13)	1% (5.47)	36% (38.06)	53% (46.23)	4% (11.54)	3% (9.98)	1% (5.47)	39% (38.65)	54% (47.29)	4% (11.54)	3% (9.98)	0% (0)
4	39% (38.05)	50% (45.00)	3% (9.98)	3% (9.98)	1% (5.47)	40% (39.23)	50% (45.00)	3% (9.90)	4% (11.54)	1% (5.47)	38% (38.06)	52% (46.15)	3% (9.98)	3% (9.98)	0% (0)	41% (39.60)	51% (45.57)	3% (9.98)	3% (9.98)	0% (0)	37% (37.47)	52% (46.15)	3% (9.98)	4% (11.54)	1% (5.47)	38% (38.06)	51% (45.57)	3% (9.98)	3% (9.98)	0% (0)
5	37% (37.47)	54% (47.29)	3% (9.98)	2% (8.43)	1% (57)	38% (38.06)	55% (47.87)	4% (11.54)	3% (9.98)	0% (0)	39% (38.65)	52% (46.15)	3% (9.98)	3% (9.98)	1% (5.47)	40% (39.23)	54% (47.29)	4% (11.54)	2% (8.13)	0% (0)	40% (39.23)	54% (47.29)	3% (9.98)	3% (9.98)	0% (0)	42% (40.40)	55% (47.87)	4% (11.54)	3% (9.98)	1% (5.47)
Mean ± S.E.M	37.46 ± 0.42	47.75 ± 0.55	9.07 ± 0.90	8.87 ± 0.45	3.28 ± 1.33	37.81 ± 0.54	46.27 ± 0.58	10.23 ± 0.63	10.14 ± 0.74	4.37 ± 1.09	37.69 ± 0.83	46.49 ± 0.34	9.98 ± 0	11.19 ± 0.55	2.18 ± 1.33	38.8 ± 0.51	46.43 ± 0.44	8.78 ± 1.16	9.03 ± 0.79	2.18 ± 1.33	38.6 ± 0.82	46.7 ± 0.34	9.5 ± 0.64	10.4 ± 0.38	3.28 ± 1.33	39.34 ± 0.85	47.41 ± 0.44	10.6 ± 0.38	10.5 ± 0.58	3.2 ± 1.33
Geo mean	36.97	53.16	2.4	2.35	0	37.55	52.35	3.1	3.28	0	37.28	52.58	3	3.72	0	39.35	52.38	2.35	2.55	0	38.9	53.39	2.7	3.56	0	40.09	54.17	3.36	3.32	0

N = Neutrophil
L = Lymphocyte
M = Monocyte
E = Eosinophil
B = Basophil

The data in paranthesis indicate are sin value.

31

difference from the value obtained at 4, 8, 12, 24 and 48 h (Table 8A). Mean ± S.E.M. value of 7.40 ± 0.11, 7.39 ± 0.11, 6.64 ± 0.07, 6.12 ± 0.04 and 6.36 ± 0.07 g/dl were obtained at 4, 8, 12, 24 and 48 h. post s.c. injection of ivermectin.

Table – 8

Effect of single s.c. injection of ivermectin (0.66 mg/kg) at various time intervals in goats on total plasma protein (g/dl).

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	7.2	7.4	7.6	6.6	6.2	6.9
2	7.0	7.2	7.4	6.8	6.0	6.2
3	7.4	7.6	7.2	6.6	6.2	6.6
4	7.8	7.6	7.4	6.8	6.0	6.2
5	7.6	7.4	7.0	6.4	6.2	6.4
Mean ± S.E.M.	7.40 ± 0.13	7.40 ± 0.11	7.34 ± 0.11	6.64 ± 0.07	6.12 ± 0.04	6.36 ± 0.07

Table – 8A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F	C.S.S.	M.S.	F value
Between hours	5	8.21	1.64	0.08[NS]
Error	24	1.2	20	

[NS] = Non significant

(ii)   Blood Glucose :

Table 9 and Fig. 2 depict the values of blood glucose before and after single s.c. injection of ivermectin (0.66 mg/kg). Mean ± S.E.M. value of 56.2 ± 0.58 mg/dl was obtained at 0 h. Mean value of 56.4 ± 0.56, 57 ± 0.31, 56.2 ± 0.73, 57.4 ± 0.24 and 55.8 ± 0.58 mg/dl were obtained at 4, 8, 12, 24 and 48 h after injection of ivermectin. Analysis of data by single factor ANOVA reveals non significant difference (Table 9A) which denotes that single injection of ivermectin does not have any significant effect on blood glucose.

Table – 9

Effect of single s.c. injection of ivermectin (0.66 mg/kg) at various time intervals in goats on blood glucose level (mg/dl)

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	55	55	56	57	57	57
2	57	58	58	55	58	56
3	55	56	57	57	57	57
4	56	56	57	57	57	57
5	58	58	57	58	58	55
Mean ± S.E.M.	56.2 ± 0.58	56.4 ± 0.56	57 ± 0.31	56.2 ± 0.73	57.4 ± 0.24	55.8 ± 0.58

Table – 9A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F	C.S.S.	M.S.	F
Between hours	5	8.7	1.74	1.2^{NS}
Error	24	34.8	1.45	

^{NS} = Non significant

(iii) *Serum cholesterol*

Effect of single s.c. injection of ivermectin (0.66 mg/kg) at various time intervals in goat on serum cholesterol is shown in Table 10 and Fig. 3. Mean ± S.E.M. value of 53.4 ± 0.74 mg/dl was obtained at 0 h which did not differ significantly from that of 4 h (54.4 ± 0.74 mg/dl) 8 h (51.1 ± 0.65 mg/dl), 12 h (56.2 ± 0.58 mg/dl), 24 h (57 ± 0.63 mg/dl) and 48 h (55 ± 0.54 mg/dl). The result shows that single s.c. injection of ivermectin does not have any significant effect on serum cholesterol (Table 10 A).

Table – 10

Effect of single s.c. injection of ivermectin (0.66 mg/kg) at various time intervals in goats on serum cholesterol (mg/dl)

Animal No.	Time in Hours					
	0	**4**	**8**	**12**	**24**	**48**
1	52	53	54	55	56	54
2	53	54	55	56	56	55
3	52	53	54	55	56	54
4	54	55	56	57	58	55
5	56	57	58	58	59	57
Mean ± S.E.M.	53.4 ± 0.74	54.4 ± 0.74	51.1 ± 0.65	56.2 ± 0.58	57.0 ± 0.63	55.0 ± 0.54

Table – 10 A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F	C.S.S.	M.S.	F
Between hours	5	48.97	9.79	0.82[NS]
Error	24	267.2	11.13	

[NS] = Non-significant

(iv) Blood urea nitrogen (BUN) :

Table 11 and Fig. 4 present the value of BUN at 0 h before administration and at 4, 8, 12, 24, and 48 h post single s.c. injection of ivermectin (0.66 mg/kg). Mean ± S.E.M value of 13.2 ± 0.37 mg/dl was obtained before administration of the drug (0 h), which did not differ significantly from that of 4, 8, 12, 24 and 48 hr post s.c. injection of ivermectin (Table 11A).

Table – 11

Effect of single s.c. injection of ivermectin (0.66 mg/kg) at various time intervals in goats on blood urea nitrogen (BUN in mg/dl).

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	14	13	12	13	14	14
2	13	12	13	14	13	13
3	12	13	14	13	12	12
4	14	13	12	13	14	14
5	13	12	13	14	13	13
Mean ± S.E.M.	13.2 ± 0.37	12.6 ± 0.24	12.8 ± 0.37	13.4 ± 0.24	13.2 ± 0.37	13.2 ± 0.37

Table – 11A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F	C.S.S.	M.S.	F
Between hours	5	2.27	0.454	0.802[NS]
Error	24	13.60	0.566	

[NS] = Non significant

(v)   Serum glutamate pyruvate transaminase (SGPT)

Table 12 and Fig. 5 present the values of SGPT at 0 h (before drug administration) and at 4, 8, 12, 24 and 48 h post single s.c. injection on of ivermectin (0.66 mg/kg). Mean ± S.E.M. value of 31.2 ± 0.48 IU/L was obtained before administration of the drug (0 h), which did not differ significantly from that of 4, 8,12, 24, and 48 h post s.c injection of ivermectin (Table 12 A).

Table – 12

Effect of single s.c. injection of ivermectin (0.66 mg/kg) at various time intervals in goats on SGPT.

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	32	30	34	36	40	38
2	32	32	32	34	36	38
3	30	32	34	35	40	40
4	32	32	34	36	38	38
5	30	33	34	36	37	38
Mean ± S.E.M.	31.2 ± 0.48	31.8 ± 0.48	33.6 ± 0.48	35.4 ± 0.4	38.2 ± 0.44	38 ± 0.44

Table – 12A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F	C.S.S.	M.S.	F
Between hours	5	193.9	38.78	1.91[NS]
Error	24	485.6	20.23	

[NS] = Non significant

(vi) Serum glutamate oxaloacetate transaminase (SGOT)

Effect of single S.C. injection of ivermectin (0.66 mg/kg) at various time intervals in goats on SGOT is presented in Table 13 and Fig. 6. A Mean ± S.E.M of 19.4 ± 0.79 IU/L was obtained before administration of the drug, which increased to 20.6 ± 0.67, 21.8 ± 0.66, 23.8 ± 0.66, 25.8 ± 0.57 and 26.8 ± 0.58 IU/L at 4, 8, 12, 24, and 48 h, respectively, post S.C. injection of ivermectin. Analysis of data by single factor ANOVA denotes that there is a significant increase between 12, 24 and 48 h, but non-significant difference is noted between 4, 8, 12 hr. Maximum value was obtained at 48 h (26.8 ± 0.58 IU/L).

Table – 13

Effect of single s.c. injection of ivermectin (0.66 mg/kg) at various time intervals in goats on SGOT.

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	18	20	22	24	26	28
2	19	20	21	23	25	27
3	20	21	22	24	26	26
4	18	19	20	22	24	25
5	22	23	24	26	28	28
Mean ± SEM	19.4^a ± 0.74	20.6^a ± 0.67	21.8^a ± 0.66	23.8^b± 0.66	25.8^b ± 0.57	26.8^b ± 0.58

Showing analysis of data by single factor ANOVA

Sources of variation	D.F	C.S.S.	M.S.	F
Between hours	5	215.37	43.07	19.31*
Error	24	53.6	2.23	

*p < 0.05

II. TOXICITY STUDIES AFTER A SINGLE HIGHER DOSE (3.3 mg/kg. S.C)

Toxicity studies after a single higher dose i.e. 5 times the therapeutic dose (3.3 mg/kg s.c.) of ivermectin were conducted in each of 5 healthy goats. The blood samples were collected at 0 h. (before administration of drug) and 4, 8, 12, 24 & 48 h. post drug administration. The following parameters were studied and the results were noted below.

A. CLINICAL PARAMETERS :

(i) Respiration rate :

Effect of single s.c. injection of ivermectin (3.3 mg/kg) on respiration rate at different time is present in Table - 14. The values of respiration rate/min at 0 h ranged from 18-22 with a mean of 20.8 ± 0.86. Analysis of data shows non-significant difference between the

value noted at 0 h and 4, 8, 12. 24 & 48 h post drug administration. This clearly shows that drug has no significant effect on respiration rate.

Table – 14

Effect of single s.c. injection of ivermectin at five times the therapeutic dose (3.3 mg/kg) at various times intervals in goats on respiratory rate/min.

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	22	25	26	28	28	30
2	20	22	24	26	27	28
3	18	20	21	18	19	22
4	23	20	22	24	26	25
5	21	23	19	22	24	24
Mean ± S.E.M.	20.8 ± 0.82	21.4 ± 0.89	22.4 ± 1.20	23.0 ± 1.86	24.8 ± 1.59	25.3 ± 1.44

Table – 14A

Showing analysis of data by single factor ANOVA

Sources of variation	D. F.	C. S .S	M. S.	F
Between Days	5	86.57	17.31	1.954[NS]
Error	24	212.8	8.86	

[NS] = Non significant.

(ii) **Pulse Rate :**

Table 15 presents the value of single s.c. injection of ivermectin (3.3 mg/kg) on pulse rate. Mean ± S.E.M. value of 72.80 ± 0.86/min was noted at 0 h which slightly increased to 74.00 ± 1.22, 74.70 ± 1.51, 75.65 ± 1.77, 76.44 ± 1.98 and 76.90 ± 2.14/min at 4, 8, 12, 24 and 48 h, respectively. Analysis of data by single factor ANOVA (Table 15A) does not show any significant difference. Thus, it seems that ivermectin may not have any effect on pulse rate.

Table – 15

Effect of single s.c. injection of ivermectin at the five times therapeutic dose (3.3 mg/kg) at various time intervals in goats on pulse rate/min.

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	72	75	76	80	80	82
2	73	73	75	78	82	80
3	74	76	78	77	75	72
4	70	72	70	72	74	75
5	75	80	82	85	87	88
Mean ± S.E.M.	72.80 ± 0.86	74.00 ± 1.22	74.70 ± 1.51	75.65 ± 1.77	76.44 ± 1.99	76.90 ± 2.14

Table – 15A
Showing analysis of data by single factor ANOVA

Sources of variation	D. F.	C. S .S	M. S.	F
Between Days	5	179.87	35.97	1.769[NS]
Error	24	488	20.33	

[NS] = Non significant.

(iii) Body Temperature :

Table – 16 presents the value of single s.c. injection of ivermectin (3.3 mg/kg) on body temperature. Mean ± S.E.M value of 102.88 ± 0.29°F was noted at 0 h which increased non significantly to 102.3 ± 0.40, 103.4 ± 0.34, 0.34, 103.5 ± 0.31, 103.9 ± 0.29 and 103.8 ± 0.20°F at 4, 8, 12, 24 & 48 h at post administration of drug.

Table – 16

Effect of single s.c. injection of ivermectin at the five time therapeutic dose (3.3 mg/kg) at various times intervals in goats on body temperature (°F)

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	103.0	103.4	103.8	103.4	104	104.2
2	102.6	102.6	102.4	103	103.4	103
3	102.0	102.0	103.0	103.2	103.4	103.8
4	103.8	104.2	103.4	104.8	105.0	104.0
5	103.0	103.8	104.4	103.4	103.8	104.0
Mean ± S.E.M.	102.98 ± 0.29	103.2 ± 0.40	103.4 ± 0.34	103.5 ± 0.31	103.4 ± 0.29	103.8 ± 0.20

Table –17A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C. S .S	M. S.	F
Between Days	5	3.7	0.74	0.474 NS
Error	24	37.677	1.56	

NS = Non significant.

B. HAEMATOLOGICAL PARAMETERS

(i) Hemoglobin (Hb in g/dl) :

Different values of haemoglobin noted at different times (0, 4, 8, 12, 24 and 48 h) after single s.c. injection of ivermectin are presented in Table 18. The value of haemoglobin before drug administration (0 h) varied from 8.2-10.6 gm/dl with a Mean ± S.E.M 9.46 ± 0.41 gm/dl. Mean ± S.E.M values of 9.42 ± 0.37, 9.37 ± 0.35, 9.33 ± 0.35, 9.32 ± 0.35 and 9.35 ± 0.35 gm/dl were noted of 4, 8, 12, 24 and 48 h, respectively. Analysis of data shows non significant difference between hours. (Table 18A). This indicates that ivermectin has no significant effect on haemoglobin.

Table – 18

Effect of single s.c. injection of ivermectin at five times the therapeutic dose (3.3 mg/kg) at various time intervals in goats on Hemoglobin (Hb in g/dl)

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	10.6	10.4	10.4	10.8	10	10.4
2	10.0	9.6	9.4	9.0	9.8	10.0
3	8.9	8.6	8.3	8.5	8.8	9.0
4	8.2	8.5	8.9	8.7	8.1	8.2
5	9.6	9.8	9.4	9.0	9.8	10.0
Mean ± S.E.M.	9.46 ± 0.41	9.42 ± 0.37	9.37 ± 0.35	9.33 ± 0.35	9.32 ± 0.35	9.35 ± 0.35

Table -18 A

Table -18 A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C. S .S	M. S.	F
Between Days	5	0.35	0.07	0.094^{NS}
Error	24	17.87	0.74	

^{NS} = Non significant.

(ii) Total leucocyte count (T.L.C.)

Effect of ivermectin on total leucocyte count (TLC) after single s.c. injection (3.3 mg/kg) is shown in Table 19. At 0 h, mean ± S.E.M. of TLC was noted to be 10124 ± 231.7/mm^3 of blood, which non significantly decreased to 10058 ± 192.5, 10049.8 ± 182.3, 10052.25 ± 178.06, 10056.64 ± 175.20 and 10062.03 ± 172.86 at 4, 8, 12, 24 and 48 h, respectively, post s.c. injection. Analysis of data by ANOVA reveals non-significant difference between hours (Table 19A). It seems that ivermectin has no significant effect on TLC.

Table – 19

Effect of single s.c. injection of ivermectin at the five time of therapeutic dose (3.3 mg/kg) at various times intervals in goats on total leucocyte count (T.L.C.)

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	10875	10250	10425	10500	10550	10555
2	9625	9630	9645	9700	9750	9800
3	9540	9545	9550	9545	9555	9560
4	10250	10300	10310	10212	10280	10300
5	10230	10235	10238	10240	10236	10230
Mean ± S.E.M.	10124 ± 231.7	10058 ± 192.5	10049.8 ± 182.3	10052.25 ± 178.06	10056.04 ± 175.20	10062.03 ± 172.86

Table –19 A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C. S .S	M. S.	F
Between Days	5	41448.8	8289.76	0.045^{NS}
Error	24	4385589.2	182732.8	

^{NS} = Non significant.

(iii) *Differential leucocyte count (D.L.C) :*

DLC noted at different hours (0, 4, 8, 12, 24 & 48 h) after single s.c. injection of ivermectin (3.3 mg/kg) are presented in Table 20. In parenthesis, the original values converted to are sin values are presented for proper statistical analysis. Single factor ANOVA reveals that there is non significant effect of ivermectin on different leucocyte viz; neutrophil, lymphocyte, monocyte, cosinophil and basophil.

Table - 20

Effect of single s.c. injection of ivermectin at the 5 times therapeutic dose (3.3 mg/kg) at various intervals in goats on differential leucocyte count (D.L.C.)

Time In Hour

Animal Number	0					4					8					12					24					48				
	N	L	M	E	B	N	L	M	E	B	N	L	M	E	B	N	L	M	E	B	N	L	M	E	B	N	L	M	E	B
1	30% (33.21)	55% (47.87)	2% (8.13)	4% (11.54)	1% (5.47)	32% (34.45)	58% (49.60)	3% (9.98)	3% (9.98)	0% (0)	35% (36.27)	60% (50.77)	3% (9.98)	4% (11.54)	5% (12.92)	37% (37.47)	60% (50.77)	2% (8.13)	4% (11.54)	1% (5.47)	39% (38.65)	62% (51.94)	3% (9.98)	4% (11.54)	1% (5.47)	32% (34.45)	60% (50.77)	5% (12.92)	4% (11.54)	1% (5.47)
2	35% (36.27)	52% (46.15)	3% (9.98)	4% (11.54)	0% (0)	37% (37.47)	54% (47.29)	4% (11.54)	2% (8.13)	1% (5.47)	38% (38.06)	55% (47.87)	3% (9.98)	4% (11.54)	0% (0)	40% (39.23)	56% (48.45)	3% (9.98)	5% (12.42)	1% (5.47)	42% (40.44)	56% (48.60)	4% (11.54)	4% (11.54)	0% (0)	32% (33.21)	59% (50.10)	4% (11.54)	5% (12.92)	1% (5.47)
3	37% (37.47)	55% (47.87)	4% (11.54)	5% (12.92)	0% (0)	39% (38.65)	54% (47.29)	4% (11.54)	4% (11.54)	5% (5.47)	40% (39.23)	55% (47.87)	4% (11.54)	5% (12.42)	0% (0)	42% (40.44)	54% (47.27)	5% (9.98)	4% (11.54)	0% (0)	44% (41.55)	52% (46.15)	2% (8.13)	4% (11.54)	1% (5.47)	37% (37.47)	53% (46.72)	3% (9.98)	4% (11.54)	0% (0)
4	32% (34.45)	54% (47.29)	3% (9.98)	5% (12.92)	0% (0)	34% (35.67)	56% (48.45)	4% (11.54)	5% (12.92)	0% (0)	36% (36.87)	58% (49.60)	3% (8.13)	4% (11.54)	0% (0)	38% (38.06)	60% (50.77)	4% (11.54)	5% (12.92)	1% (5.47)	41% (39.82)	62% (51.94)	3% (9.98)	4% (11.54)	1% (5.47)	35% (36.27)	60% (50.77)	3% (9.98)	3% (9.98)	1% (5.47)
5	37% (38.65)	54% (49.00)	2% (8.13)	3% (9.98)	1% (5.47)	41% (34.82)	59% (50.10)	3% (9.98)	4% (11.54)	0% (0)	43% (40.98)	62% (51.44)	3% (8.13)	5% (12.08)	1% (5.47)	45% (42.13)	64% (53.13)	4% (11.54)	5% (12.42)	1% (5.47)	45% (42.13)	65% (53.73)	3% (9.98)	3% (9.98)	1% (5.47)	30% (38.05)	60% (54.33)	4% (11.54)	4% (11.54)	0% (0)
Mean ± SEM	36.01± 0.98	47.75± 0.55	9.55± 0.64	11.17± 0.61	2.18± 1.33	37.2± 0.97	48.50± 0.58	10.9± 0.38	10.8± 0.81	2.18± 1.33	38.28± 0.84	48.49± 1.55	10.2± 0.31	12.09± 0.33	2.18± 1.33	39.46± 0.83	50.08± 1.01	10.24± 0.32	11.14± 0.70	4.37± 1.09	40.51± 0.61	50.67± 1.30	9.42± 0.54	11.22± 0.31	4.37± 1.09	36.64± 0.98	50.55± 1.20	11.19± 0.55	11.50± 0.46	3.28± 1.33
Geo mean	34.44	54.76	2.7	3.72	0	36.45	56.16	3.56	3.43	0	38.29	57.93	3.17	4.37	0	40.29	58.59	3.1	4.57	0	42.14	59.62	2.93	3.77	0	34.44	59.45	3.72	3.94	0

N = Neutrophil

L = Lymphocyte

M = Monocyte

E = Eosinophil

B = Basophil

The data in parenthesis indicate are sin value.

Table – 20A

Analysis of data by single factor ANOVA showing effect of single s.c. injection of ivermectin at five times the therapeutic dose (3.3 mg/kg) at various times intervals in goats on differential leucocyte to count (DLC)

Leucocytes	Source of variation	D.F.	C.S.S.	M.S.	F
Neutrophil	Between Hour	5	85.32	17.06	7.40
	Error	24	93.10	3.57	
Lymphocyte	Between Hour	5	34.11	6.82	1.48
	Error	24	111.15	4.58	
Monocyte	Between Hour	5	9.39	1.87	1.36
	Error	24	33.14	1.38	
Eosinophil	Between Hour	5	8.03	1.60	1.28
	Error	24	30.22	1.25	
Burophil	Between Hour	5	28.89	5.77	0.72
	Error	24	191.53	7.90	

NS = Non-significant

C.   BIOCHEMICAL PARAMETERS :

(i)   Total plasma protein :

Table 21 and Fig. 7 present the values of total plasma protein before (0 h) and 4, 8, 12, 24 & 48 h post single s.c. injection of ivermectin (3.3 mg/kg). At 0 h, a mean ± S.E.M. value of 7.18 ± 0.14 g/dl was estimated as total plasma protein value in goats which does not show any significant difference from the values obtained at 4, 8, 12, 24 and 48 h (Table 21 and 21 A). Mean ± S.E.M value of 7.21

± 0.11, 7.28 ± 0.11, 7.31 ± 0.10, 7.31 ± 0.10 and 7.30 ± 0.10 g/d were obtained 4, 8, 12, 24 and 48 h post injection of ivermectin.

Table – 21

Effect of single s.c. injection of ivermectin at five times the therapeutic dose (3.3 mg/kg) at various time intervals in goats on total plasma protein.

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	7.6	7.2	7.8	7.4	7.7	7.5
2	7.1	7.3	7.5	7.6	7.2	7.3
3	7.0	7.2	7.4	7.1	7.4	7.2
4	6.8	7.0	7.2	7.6	7.2	7.3
5	7.4	7.5	7.3	7.2	7.0	7.1
Mean ± S.E.M.	7.18 ± 0.17	7.21 ± 0.11	7.28 ± 0.11	7.31 ± 0.10	7.31 ± 0.10	7.30 ± 0.30

Table – 21 A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Days	5	0.2	0.04	0.714[NS]
Error	24	1.35	0.056	

[NS] = Non significant.

(ii)   Blood glucose :

Table 22 and Fig. 8 depict the values of blood glucose before and after s.c. injection of ivermectin (3.3 mg/kg s.c.). Mean ± S.E.M. value of 85.10 ± 2.70 mg/dl was obtained at 0 h. Mean values of 85.43 ± 2.17, 85.18 ± 2.07, 86.19 ± 197, 86.42 ± 1.83 and 86.53 ± 1.71 mg/dl were obtained at 4, 8, 12, 24 and 48 h after s.c injection of ivermectin. Analysis of data by single factors ANOVA reveals non-significant difference (Table 22A) which denotes that single s.c. injection of ivermectin at higher dose does not have any significant effect on blood glucose.

Table – 22

Effect of single s.c. injection of ivermectin at five times the therapeutic dose (3.3 mg/kg) at various time intervals in goats on blood glucose

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	75.0	78.0	80.0	82.0	85.0	85.9
2	85.5	85.2	85.5	86.0	85.7	85.0
3	86.0	86.2	86.2	87.0	87.0	86.2
4	88.0	88.5	90.5	90.8	87.7	87.7
5	91.0	90.3	91.1	91.3	91.5	90.8
Mean ± S.E.M.	85.10 ± 2.70	85.43 ± 2.17	85.80 ± 2.07	86.19 ± 1.97	86.42 ± 1.83	86.53 ± 1.71

Table – 22 A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Days	5	23.59	4.71	0.270[NS]
Error	24	418.82	17.450	

[NS] = Non significant.

(iii) Serum cholesterol

Effect of single s.c. injection of ivermectin (3.3 mg/kg) at various time interval in goats on serum cholesterol is shown in Table 23 and Fig 9. Mean ± S.E.M. value of 53.32 ± 0.70 mg/dl was obtained at 0 h which did not differ significantly from that of 4 h (54.09 ± 0.75 mg/dl), 8 h (56.02 ± 0.54 mg/dl), 12 h (56.58 ± 0.58 mg/dl) 24 h (57.06 ± 0.70 mg/dl) and 48 h (56.71 ± 0.75 mg/dl). The result shows that single s.c. injection of ivermectin does not have any significant effect on serum cholesterol.

Table – 23

Effect of single s.c. injection of ivermectin at five times the therapeutic dose (3.3 mg/kg) at various time intervals in goats on serum cholesterol

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	51.30	53.05	55.80	58.00	60.00	58.00
2	53.20	55.00	56.10	57.50	58.00	56.00
3	54.50	55.80	56.20	57.10	57.90	56.30
4	52.40	53.60	54.30	55.00	55.30	53.10
5	55.20	56.90	57.70	58.10	58.90	55.00
Mean ± S.E.M.	53.32 ± 0.70	54.09 ± 0.75	56.02 ± 0.54	56.58 ± 0.58	57.06 ± 0.70	56.71 ± 0.75

Table-23 A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Days	5	68.85	13.77	0.096[NS]
Error	24	3409.65	142.06	

NS = Non significant

(iv) Blood urea nitrogen (BUN)

Table 24 and Fig. 10 present the values of blood urea nitrogen (BUN) at 0 h (before administration) and at 4, 8, 12, 24 & 48 h post single s.c. injection of ivermectin (3.3 mg/kg). Mean ± S.E.M value of 13.51 ± 0.31 mg/dl was obtained before administration of the drug (0 h), which did not differ significantly from that of 4, 8, 12, 24 and 48 h post s.c. injection of ivermectin (Table 24A).

Table – 24

Effect of single s.c. injection of ivermectin at five time the therapeutic dose (3.3 mg/kg) at various times intervals in goats on BUN

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	14.00	14.20	14.00	13.90	14.80	14.00
2	13.40	13.28	13.75	13.80	14.00	13.30
3	12.90	13.10	13.40	12.58	13.00	12.80
4	14.45	13.80	12.20	12.48	12.80	13.00
5	12.80	13.60	12.25	13.70	13.72	13.75
Mean ± S.E.M.	13.51 ± 0.31	13.55 ± 0.24	13.40 ± 0.29	13.37 ± 0.29	13.43 ± 0.30	13.37 ± 0.22

Table – 24 A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Days	5	0.874	0.174	0.3703^{NS}
Error	24	11.34	0.472	

^{NS} = Non significant.

(v) Serum glutamate pyruvate transaminase (SGPT)

Table 25 and Fig. 11 depict the values of SGPT at 0 h (before administration) and at 4, 8, 12, 24 & 48 h. post single s.c. injection of ivermectin (3.3 mg/kg). Mean $\pm$ S.E.M. value of 27.24 $\pm$ 1.39 IU/L was obtained before administration of the drug (0 h) which did not differ significantly from that of 4, 8, 12, 24 and 48 h post s.c. injection of ivermectin (Table 25A).

Table – 25

Effect of single s.c. injection of ivermectin at five time the therapeutic dose (3.3 mg/kg) at various times intervals in goats on Serum Glutanate pyravate Transaminase (SGPT).

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	25.05	28.10	30.19	32.23	34.90	40
2	30.15	32.05	35.42	37.30	38.51	42.10
3	23.04	23.90	25	28	30.20	36
4	28	28.10	30.20	33.25	36	39
5	30	30.20	32.05	34.45	35	39.20
Mean ± S.E.M.	27.24 ± 1.39	28.47 ± 1.35	29.52 ± 1.52	30.6 ± 1.65	31.75 ± 1.76	39.26 ± 0.98

Table-25A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Days	5	535.51	107.10	3.526[NS]
Error	24	729.04	30.37	

[NS] = Non significant.

(vi) **Serum glutamatic oxaloacetate transaminases (SGOT) :**

Effect of single s.c. injection (3.3 mg/kg) of ivermectin at various time intervals in goats on SGOT is presented in Table 26 and Fig. 12. A Mean ± S.E.M 17.8 ± 1.34 IU/L was obtained before administration of the drug, which increased to 18.45 ± 1.24, 20.51 1.12, 22.33 ± 1.31, 26.01 ± 1.10 and 27.90 ± 0.93 IU/L at 4, 8, 12, 24 & 48 h respectively, post s.c. injection of ivermectin. Analysis of data by single factor ANOVA denotes that there is a significant increase at 48 h but non significant difference noted between 4, 8, 12 & 14 h . Maximum value was obtained at 48 h (27.90 ± 0.93 IU/L).

Table – 26

Effect of single s.c. injection of ivermectin at five time the therapeutic dose (3.3 mg/kg) at various times intervals in goats on serum glutamic oxaloacetate transaminase (SGOT).

Animal No.	Time in Hours					
	0	4	8	12	24	48
1	15	16.05	16.45	18	22	26
2	15	17	20	24	26	28
3	17	17.10	21.12	25.05	28	30
4	20	21.35	22	24	26	28.42
5	22	22.42	23	25.15	28.05	30.25
Mean ± S.E.M.	17.8^a ± 1.34	18.45^a ± 1.12	20.51^a ± 1.12	22.53^a ± 1.31	26.01^a ± 1.10	27.90^b ± 0.93

Different superscript differ significantly ($p < 0.05$).

Table-26A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Days	5	438.68	87.73	9.536 **
Error	24	221.02	9.20	

**P < 0.01

III. TOXICITY STUDIES OF IVERMECTIN FOLLOWING DAILY REPEATED THERAPEUTIC DOSE (0.66 mg/kg, s.c) FOR 14 DAYS

Long-term effect study of ivermectin was conducted post s.c. administration of 0.66 mg/kg once daily for 14 days in each of five healthy goats. The blood samples were collected on day 0 (before administration of the drug) and 1, 2, 4, 8 & 14 day post drug administration. The following parameters were studied and the results were noted below.

(A) CLINICAL PARAMETERS :

(i) Respiration rate :

Effect of single s.c. injection of 0.66 mg/kg body weight of ivermectin on respiration rate at different days is presented in Table 27. The value of respiration rate/min on day 0 ranged from 18-25 with a mean of 21.6 ± 1.2/min. Analysis of data shows non-significant

difference between the values noted on day 0 (pretreatment) and on day 1, 2, 4, 8 and 14 post drug administration (Table 27A). This clearly shows that the drug has no effect on respiration rate.

Table – 27
Effect of daily s.c. injection of ivermectin at the therapeutic dose (0.66 mg/kg) for 14 days on various intervals of days in goats on Respiratory rate/min.

Animal No.	Time in Days					
	0	1	2	4	8	14
1	18	20	21	22	24	22
2	20	22	24	26	22	25
3	22	24	23	25	24	23
4	25	23	20	22	21	24
5	23	25	26	24	21	20
Mean ± S.E.M.	21.6 ± 1.20	22.2 ± 1.00	22.4 ± 1.01	23.8 ± 0.80	23.2 ± 0.72	23.06 ± 0.74

Table – 27A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Days	5	12.27	2.454	0.580[NS]
Error	24	101.6	4.23	

[NS] = Non significant.

(ii) Pulse Rate :

Table 28 presents the effect of s.c. injection of ivermectin (0.66 mg/kg) once daily for 14 days on pulse rate. Mean ± S.E.M. value of 75.6 ± 1.5/min was noted on day 0 which slightly increased to 76.5 ± 1.41, 77.2 ± 1.38, 77.5 ± 1.49, 78.4 ± 1.52 and 78.7 ± 1.51/min respectively. Analysis of data by single factor ANOVA (Table 28A) does not show any significant difference. Thus, it seems that ivermectin may not have any effect on pulse rate.

Table – 28

Effect of daily s.c.. injection of ivermectin at the therapeutic dose (0.66 mg/kg) for 14 days on various intervals of day in goats on pulse rate/min.

Animal No.	Time in Days					
	0	1	2	4	8	14
1	78	80	79	82	80	78
2	80	81	82	85	86	85
3	73	75	77	78	80	81
4	75	7	80	79	78	77
5	72	74	75	77	77	80
Mean ± S.E.M.	75.6 ± 1.50	76.5 ± 1.41	77.2 ± 1.38	77.5 ± 1.49	78.4 ± 1.52	78.7 ± 1.51

Table – 28 A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Days	5	90.3	18.06	1.79 NS
Error	24	242	10.08	

NS = Non significant.

(iii) Body temperature :

Effect of s.c. injection of ivermectin (0.66 mg/kg) daily for 14 days on body temperature is presented in Table 29. Mean ± S.E.M. value of 102.76 ± 0.23° F was noted on day 0 which increased slightly to 102.84 ±   0.17° F, 102.76 ± 0.30° F, 102.74 ± 0.29° F, 102.76 ± 0.30 and 103.24 ± 0.11° F on day 1, 2, 4, 8 and 14 post administration of the drug. Non significant difference in body temperature is noted between days (Table 29 A) indicating that the drug may not have any effect on body temperature.

Table – 29

Effect of daily S.C. injection of ivermectin at the therapeutic dose (0.66 mg/kg) for 14 days on various intervals of days in goats on body temperature (°F).

Animal No.	Time in Days					
	0	1	2	4	8	14
1	102.8	102.6	102.0	103.0	102.0	103.0
2	103.0	103.2	103.8	103.0	102.4	103.4
3	102.0	102.8	102.6	102.0	103.0	103.2
4	103.4	103.2	103.0	103.6	103.8	136.0
5	102.6	102.8	102.4	102.0	102.6	103.0
Mean ± S.E.M.	102.76 ± 0.23	102.84 ± 0.17	102.76 ± 0.30	102.74 ± 0.29	102.76 ± 0.30	103.24 ± 0.11

Table – 29A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Days	5	0.96	0.19	0.63 [NS]
Error	24	7.38	0.30	

[NS] = Non significant.

(iv) Ruminal movement :

Table 30 presents the effect of daily s.c. injection of ivermectin (0.66 mg/kg for 14 days) on ruminal movement. On day 0 the ruminal movement/5 min ranged from 4-7 with Mean of 5.8 ± 0.58. Mean ± S.E.M. values of 5.8 ± 0.69, 6 ± 0.67, 58 ± 0.65, 5.8 ± 1.34 and 5.76 ± 0.58/5 min. were obtained on day 1, 2, 4, 8 and 14, respectively. These values do not differ significantly between days, which denote that ivermectin has not significant in ruminal movement. (Table 30A) which denotes that ivermectin may not have any significant effect on ruminal movement.

Table – 30

Effect of daily s.c. injection of ivermectin at the therapeutic dose (0.66 mg/kg) for 14 days on various intervals of days in goats on ruminal movement

Animal No.	Time in Days					
	0	1	2	4	8	14
1	4	5	8	7	5	6
2	5	6	5	4	7	4
3	7	8	6	5	6	5
4	7	3	5	4	6	7
5	6	7	8	6	5	6
Mean ± S.E.M.	5.8 ± 0.58	5.8 ± 0.69	6.0 ± 0.67	5.8 ± 0.65	5.8 ± 1.34	5.76 ± 0.50

Table – 30 A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Days	5	3.8	0.76	0.40[NS]
Error	24	45.6	1.9	

NS = Non significant.

B.   HAEMATOLOGICAL PARAMETERS :

(i)   Hemoglobin (Hb in g/dl) :

Different values of haemaglobin noted on different days (0, 1, 2. 4, 8 and 14) after daily s.c. injection of ivermectin are presented in Table 31 and Fig 19. The values of haemoglobin before drug administration (day 0) varied from 9.0 to 10.2 g/dl with a mean of 8.36 = 0.33 g/dl. Mean ± S.E.M. value of 9.48 ± 0.35, 9.50 ± 0.39, 9.54 ± 0.39, 9.60 ± 0.37 and 9.88 ± 0.24 g/dl were noted an day 1, 2, 4, 8 and 14, respectively. Analysis of data shows non-significant difference between days (Table 31A). This indicates that ivermectin has no significant effect on Hb.

Table – 31

Effect of daily s.c. injection of ivermectin at the therapeutic dose (0.66 mg/kg) for 14 days on various intervals of days in goats on hemoglobin (g/dl).

Animal No.	Time in Days					
	0	1	2	4	8	14
1	9.0	9.6	9.4	9.8	10.0	9.8
2	9.2	8.8	9	9.4	9.8	10.0
3	10.2	10.6	10.8	10	10.2	10.4
4	8.4	8.6	8.0	8.2	8.8	9.0
5	10.0	10.4	10.6	10.8	10.4	10.2
Mean ± S.E.M.	9.36 ± 0.33	9.48 ± 0.35	9.50 ± 0.39	9.54 ± 0.39	9.60 ± 0.37	9.88 ± 0.24

Table – 31 A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Days	5	4.73	0.94	1.332[NS]
Error	24	17.27	0.71	

[NS] = Non significant.

(ii) Total leucocyte count (TLC) :

Effect of ivermectin on total leucocyte count (TLC) after daily s.c. administration (0.66 mg/kg) of ivermectin is shown in Table 32. On day 0, the mean ± S.E.M. of TLC was noted to be 9858 ± 125.15/mm^3 of blood, which slightly increased to 9913.96 ± 127.4, 9918.2 ± 128.8. 9922.6 ± 131.04, 9926 ± 134.35 and 9924 ± 146. 51 mm^3 on day 1, 2, 4, 8 and 14 post s.c. injection. However, analysis of data by single factor ANOVA reveals non-significant difference between days (Table 32A). It seems that ivermectin has no significant effect on TLC.

Table – 32

Effect of daily s.c. injection of ivermectin at the therapeutic dose (0.66 mg kg) for 14 days on various intervals of days in goats on total leucocyte count.

Animal No.	Time in Days					
	0	1	2	4	8	14
1	9950	9965	9970	9972	9975	9980
2	10000	10150	10160	10200	10220	10250
3	9540	9545	9550	9555	9560	9550
4	10200	10210	10220	10222	10225	10220
5	9600	9615	9625	9630	9660	9620
Mean ± S.E.M.	9858.0 ± 125.2	9914.0 ± 127.4	9918.2 ± 128.8	9922.6 ± 131.1	9926 ± 134.4	9924 ± 146.5

Table – 32 A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Days	5	16394.2	3278.84	0.034^{NS}
Error	24	2258004.8	94083.53	

^{NS} = Non significant.

(iii) Differential leucocyte count (DLC)

Differential leucocyte count noted on different days (0, 1, 2, 4, 8 and 14) after daily s.c. injection of ivermectin (0.66 mg/kg) for the days are presented in Table 33 and Fig 21. In parenthesis the original values converted to arc sin values are presented for proper statistical analysis. Single factor ANOVA test reveals that there is no significant effect of ivermectin on differential leucocyte viz. Neutrophils, lymphocytes, monocytes, eosinophils and basophils. Statistical analysis reveals non-significant effect of ivermectin on different cells of leucocytes.

Table - 33

Effect of daily s.c. injection of ivermectin at the therapeutic dose (0.66 mg/kg) for 14 days on various time intervals in goats on D.L.C.

Time In Hour

Animal Number	0					1					2					4					8					14				
	N	L	M	E	B	N	L	M	E	B	N	L	M	E	B	N	L	M	E	B	N	L	M	E	B	N	L	M	E	B
1	35% (36.27)	54% (47.29)	2% (8.13)	3% (9.98)	1% (5.47)	37% (37.47)	50% (45.00)	3% (9.98)	1% (5.47)	0% (0)	38% (38.06)	56% (48.45)	4% (11.54)	2% (8.13)	0% (0)	39% (38.06)	54% (47.29)	3% (9.98)	3% (9.98)	0% (0)	40% (39.23)	52% (46.15)	3% (9.98)	5% (12.02)	0% (0)	42% (40.40)	53% (46.72)	4% (11.54)	2% (8.13)	0% (5.47)
2	37% (37.47)	54% (46.60)	7% (8.13)	3% (9.98)	0% (0)	39% (38.06)	55% (47.87)	4% (11.54)	3% (9.98)	1% (5.47)	40% (39.23)	57% (45.57)	5% (12.02)	3% (9.98)	1% (5.47)	41% (39.62)	56% (48.25)	5% (12.02)	2% (8.13)	1% (5.47)	43% (40.98)	54% (47.29)	4% (11.54)	3% (9.98)	1% (5.47)	40% (39.23)	52% (46.15)	3% (9.98)	2% (8.13)	1% (5.47)
3	43% (40.58)	50% (45.00)	4% (11.54)	2% (8.13)	1% (5.47)	44% (41.55)	52% (46.15)	5% (12.92)	3% (9.98)	1% (5.47)	42% (40.40)	53% (46.12)	4% (11.54)	3% (9.98)	1% (5.47)	45% (42.13)	50% (45.00)	4% (11.54)	3% (9.98)	1% (5.47)	46% (42.71)	52% (46.15)	4% (11.54)	3% (9.98)	1% (5.47)	45% (43.13)	54% (47.29)	3% (9.98)	2% (8.13)	0% (0)
4	38% (38.06)	56% (48.45)	3% (9.98)	2% (8.13)	0% (0)	40% (39.23)	55% (47.87)	4% (11.54)	3% (9.98)	0% (0)	41% (39.82)	54% (47.29)	5% (12.02)	4% (11.54)	1% (5.47)	42% (40.40)	53% (46.72)	4% (11.54)	3% (9.98)	0% (0)	44% (41.55)	50% (45.00)	5% (12.02)	4% (11.54)	0% (0)	46% (42.71)	53% (46.72)	4% (11.54)	3% (9.98)	0% (0)
5	36% (36.87)	55% (47.87)	3% (9.98)	4% (11.54)	1% (5.47)	38% (38.06)	58% (48.45)	5% (12.92)	1% (5.47)	1% (5.47)	40% (34.23)	55% (47.87)	4% (11.54)	3% (9.98)	0% (0)	42% (40.40)	54% (47.29)	5% (12.92)	4% (11.54)	0% (0)	43% (40.98)	52% (46.15)	3% (9.98)	3% (9.98)	0% (0)	42% (40.40)	53% (46.72)	3% (9.98)	2% (8.13)	0% (0)
Mean ± SEM	37.85 ± 0.74	47.6 ± 0.76	8.55 ± 0.64	9.55 ± 0.64	3.28 ± 1.33	38.99 ± 0.70	47.98 ± 0.55	11.62 ± 0.46	8.17 ± 1.10	3.28 ± 1.33	39.34 ± 0.49	47.18 ± 0.33	12.09 ± 0.33	9.92 ± 0.54	2.18 ± 1.33	40.28 ± 0.56	46.91 ± 0.53	11.78 ± 0.54	9.92 ± 0.54	4.37 ± 1.094	40.89 ± 0.58	46.14 ± 0.36	11.19 ± 0.55	10.88 ± 0.59	2.18 ± 1.33	40.47 ± 0.63	46.72 ± 0.18	10.60 ± 0.38	8.5 ± 0.37	2.18 ± 1.33
Geo mean	37.7	54.53	2.7	2.7	0	39.52	55.16	4.12	1.93	0	40.17	54.98	4.37	2.93	0	41.75	53.36	2.93	2.93	0	43.15	54.82	3.72	3.51	0	42.94	52.99	3.36	2.16	0

N = Neutrophil

L = Lymphocyte

M = Monocyte

E = Eosinophil

B = Busophil

The data in paranthesis indicate are sin value.

69

Table – 33A

Analysis of data by single factor ANOVA showing effect of single S.C. injection of ivermectin at the times therapeutic dose (0.66 mg/kg) at the 14 days on various times intervals in goats on differential leucocyte count (DLC)

Type of Leucocytes	Source of variation	D.F.	C.S.S.	M.S.	F
Neutrophil	Between Day	5	22.71	4.54	0.22[NS]
	Error	24	484.6	20.19	
Lymphocyte	Between Day	5	12.12	2.42	0.66[NS]
	Error	24	87.44	3.64	
Monocyte	Between Day	5	22.65	4.53	3.43[NS]
	Error	24	31.69	1.32	
Eosinophil	Between Day	5	25.07	5.01	2.27[NS]
	Error	24	54.22	2.2	
Basophil	Between Day	5	19.91	3.98	0.46[NS]
	Error	24	204.22	8.50	

[NS] = Non significant.

C.   BIOCHEMICAL PARAMETERS :

(i)   Total plasma protein :

Table 34 and Fig. 13 depict the values of total plasma protein before (day 0) and after (day 1, 2, 4, 8 and 14) daily s.c. injection of ivermectin (0.66 mg/kg) for 14 days in each of five goats.

On day 0, mean ± S.E.M value of 7.2 ± 0.07 gm/dl was noted as the value of total plasma protein in goats, which did not show any significant difference from the values obtained on day 1, 2, 4, 8, and 14 (Table 34 A and Fig 13). Mean ± S.E.M. value of 7.26 ± 0.06, 7.36 ± 0.09, 7.38 ± 0.09, 7.38 ± 0.09 and 7.38 ± 0.09 were obtained on day 1, 2, 4, 8 and 14 post s.c. injection of ivermectin.

Table – 34

Effect of daily s.c. injection of ivermectin at the therapeutic dose (0.66 mg/kg) for 14 days on various intervals of day in goats on total plasma protein.

Animal No.	Time in Days					
	0	1	2	4	8	14
1	7.2	7.4	7.6	7.8	7.4	7.5
2	7.4	7.4	7.8	7.4	7.6	7.2
3	7.3	7.2	7.5	7.2	7.4	7.7
4	7	7.2	7.4	7.2	7.1	7.2
5	7.1	7.4	7.6	7.5	7.6	7.6
Mean ± S.E.M.	7.2 ± 0.070	7.26 ± 0.06	7.36 ± 0.09	7.38 ± 0.09	7.38 ± 0.09	7.38 ± 0.09

Table – 34A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Days	5	1.31	0.262	0.0591[NS]
Error	24	106.47	4.43	

[NS] = Non significant.

(ii)   Blood glucose :

Table 35 and Fig. 14 present the values of blood glucose before (day 0) and after s.c. injection of ivermectin (0.66 mg/kg) on day 1, 2, 4, 8 and 14. Mean ± S.E.M. value of 80.8 ± 2.28 mg/dl was obtained on day 0. Mean values of 82.03 ± 2.06, 82.53 ± 1.92, 82.90 ± 1.89, 83.51 ± 1.85 and 83.73 ± 1.85 mg/dl were obtained on day 1, 2, 4, 8 and 14 after injection of ivermectin. Analysis of data by single factor ANOVA reveals non significant difference (Table 35 A Fig 5) between days, which denotes that injection of ivermectin may not have any significant effect on blood glucose.

Table – 35

Effect of daily S.C. injection of ivermectin at the therapeutic dose (0.66 mg/kg) for 14 days on various intervals of days in goats on Blood Glucose

Animal No.	Time in Days					
	0	1	2	4	8	14
1	77	80	82	84	86	86.1
2	75	75.5	76	76.2	77	77.2
3	80	82	84	84.1	85	85.2
4	85	85.5	85.7	86	86.1	87
5	87	87.2	87.5	88	88.1	88.7
Mean ± S.E.M.	80.8 ± 2.28	82.03 ± 2.06	82.53 ± 1.92	82.90 ± 1.89	83.51 ± 1.85	83.73 ± 1.85

Table – 35 A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Days	5	57.21	11.44	0.546[NS]
Error	24	502.09	20.92	

[NS] = Non significant.

(iii) Serum cholesterol :

Effect of daily s.c. injection of ivermectin (0.66 mg/kg on different days in goats on serum cholesterol is shown is Table 36 and Fig. 15. Mean ± S.E.M. value of 54.26 ± 0.68 mg/dl was obtained on day 0, which did not differ significantly from that of day 1 (55.38 ± 0.56), day 2 (55.92 ± 0.55), day 4 (56.28 ± 0.55), day 8 (56.28 ± 0.55) and day 14 (56.75 ± 0.65 mg/dl). The result shows that single s.c. injection of ivermectin may not have any significant effect on serum cholesterol, (Table 36 A).

Table – 36

Effect of daily s.c. injection of ivermectin at the therapeutic dose (0.66 mg/kg) for 14 days on various intervals of days in goats on serum cholesterol.

Animal No.	Time in Days					
	0	1	2	4	8	14
1	52.25	54.05	56.30	57.00	57.00	58.00
2	54.30	55.00	55.80	56.10	56.10	57.05
3	55.05	56.30	57.10	58.20	58.20	56.10
4	53.45	54.50	55.20	56.00	56.00	55.25
5	56.28	57.05	57.90	58.10	58.10	58.00
Mean ± S.E.M.	54.26 ± 0.68	55.38 ± 0.56	55.92 ± 0.55	56.28 ± 0.55	56.28 ± 0.55	56.75 ± 0.65

Table – 36 A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Days	5	32.38	6.47	
				4.46^{NS}
Error	24	34.99	1.45	

^{NS} = Non significant.

(iv) *Blood urea nitrogen (BUN)* :

Table 37 and Fig.16 present the values of BUN on day 0 (before administration) and on day 1, 2, 4, 8 and 14 post daily s.c. injection of ivermectin. (0.66 mg/kg for 14 days). Mean ± S.E.M. value of 13.91 ± 0.40 mg/dl was obtained before administration of the drug (day 0), which did not differ significantly from that of day 1, 2, 4, 8 and 14 post s.c. injection of ivermectin Table 37A and Fig 16).

Table – 37

Effect of daily s.c. injection of ivermectin at the therapeutic dose (0.66 mg/kg) for 14 days on various intervals of days in goats on blood urea nitrogen (BUN)

Animal No.	Time in Days					
	0	1	2	4	8	14
1	15.00	15.10	15.00	14.05	14.50	15.10
2	14.25	14.55	14.90	15.00	15.05	14.80
3	13.50	14.15	14.45	15.20	14.90	14.15
4	12.50	13.45	12.35	13.65	13.72	13.60
5	14.00	14.25	14.00	13.70	14.00	14.05
Mean ± S.E.M.	13.91 ± 0.40	14.1 ± 0.33	14.1 ± 0.48	14.23 ± 0.39	14.4 ± 0.25	14.3 ± 0.24

Table – 37 A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Days	5	0.87	0.174	0.299[NS]
Error	24	14.31	0.59	

[NS] = Non significant.

(v) *Serum glutamate pyruavate transaminas (SGPT) :*

Table 38 and Fig. 17 present the values of SGPT on day 0 (before administration) and on day 1, 2, 4, 8 and 14 post daily s.c. injection of ivermectin (0.66 mg/kg for 14 days) Mean ± S.E.M. value of 29.0 ± 1.49 IU/L was obtained before administration of the drug (day 0), which did not differ significantly from that of day 1, 2, 4, 8 and 14 post s.c. injection of ivermectin (Table 38 A and Fig 17).

Table – 38

Effect of daily s.c. injection of ivermectin at the therapeutic dose (0.66 mg/kg) for 14 days on various intervals of days in goats on serum glutamate pyruvate transaminur (SGPT).

Animal No.	Time in Days					
	0	1	2	4	8	14
1	26.10	27.20	29.10	31.25	33.40	36.00
2	32.25	34.05	38.40	40.32	40.82	42.20
3	25.05	25.90	26.00	28.00	28.30	29.00
4	30.00	30.25	32.10	34.05	32.00	37.00
5	32.00	32.30	35.40	36.00	36.80	38.05
Mean ± S.E.M.	29.0 ± 1.49	29.5 ± 1.43	30.4 ± 1.74	33.9 ± 2.04	34.8 ± 1.87	36.45 ± 2.14

Table – 38 A

Showing analysis of data by single factor ANOVA

Sources of variation	D.F.	C.S.S.	M.S.	F
Between Days	5	206	41.2	2.19[NS]
Error	24	451.27	18.8	

[NS] = Non significant.

(vi) Serum glutamate oxaloacetate transaminas (SGOT)

Effect of daily s.c. injection of ivermectin (0.66 mg/kg) on different days (0, 1, 2, 4, 8 and 14) is presented in Table 39 and Fig. 18. A mean value of 18.42 ± 1.35 IU/L was obtained before administration of the drug which increased to 18.92 ± 1.40 IU/L on day 1, which further increased to 19.82 ± 1.50, 20.86 ± 1.64, 25.26 ± 1.20 and 28.8 ± 1.80 IU/L on day 2, 4, 8 and 14 respectively. Analysis of data by single factor ANOVA denotes that there is a significant increase on day 8 and 14. The data shows an increasing trend with increasing days and maximum value was obtained on day 14 (28.8 ± 1.80 IU/L).

Table – 39
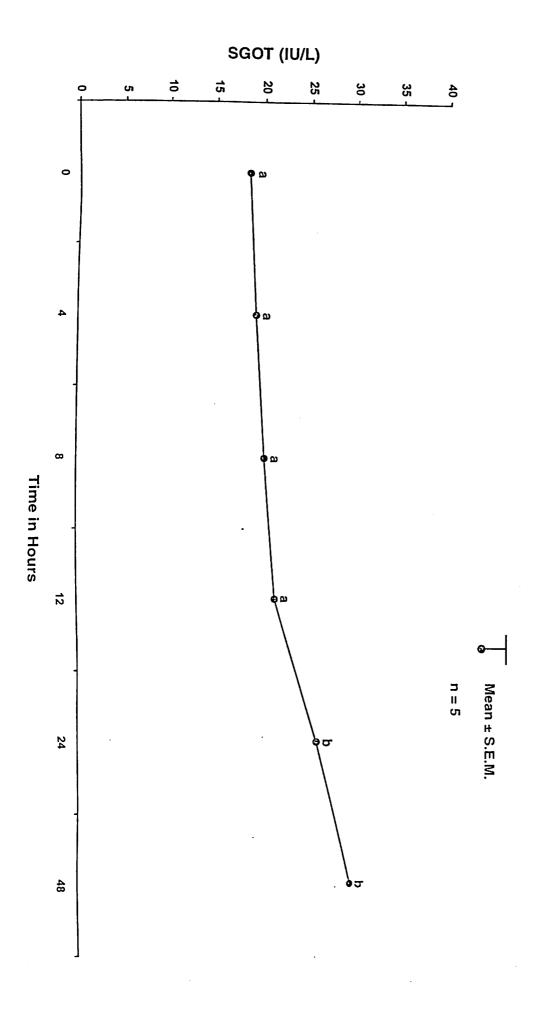
Effect of daily s.c. injection of ivermectin at the therapeutic dose (0.66 mg/kg) for 14 days on various intervals of days in goats on serum glutamate oxaloacetate transaminase (SGOT)
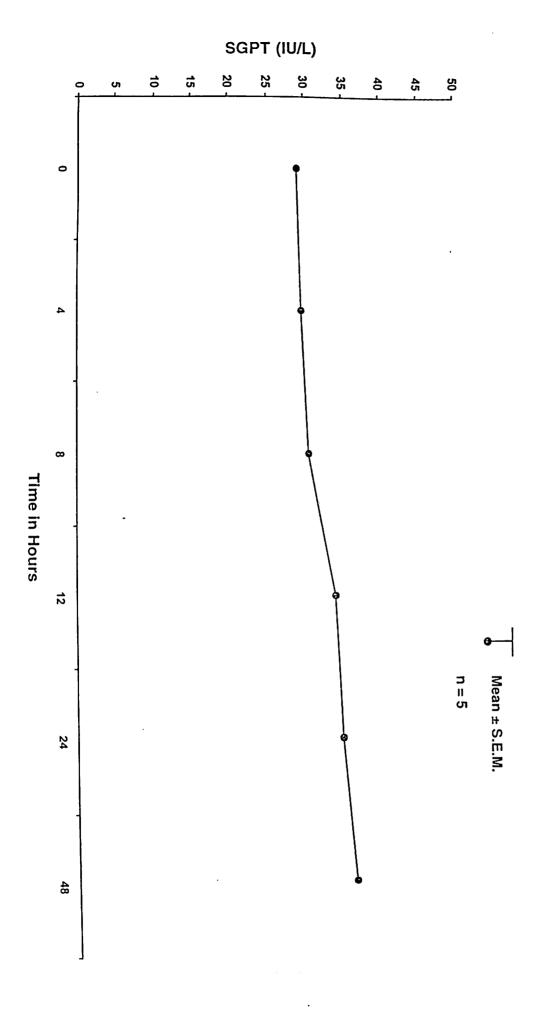
Animal No.	Time in Days					
	0	1	2	4	8	14
1	16.10	16.50	17	20.05	21	23
2	16	17	20	22	25	27
3	17	17.30	22.05	25	25.25	29
4	20	21.90	23	26.15	28.05	32.10
5	23	24.40	26.05	26.80	27	33
Mean ± S.E.M.	$18.42^a \pm$ 1.35	$18.92^a \pm$ 1.40	$19.82^a \pm$ 1.50	$20.86^a \pm$ 1.64	$25.26^b \pm$ 1.20	$28.8^b \pm$ 1.80
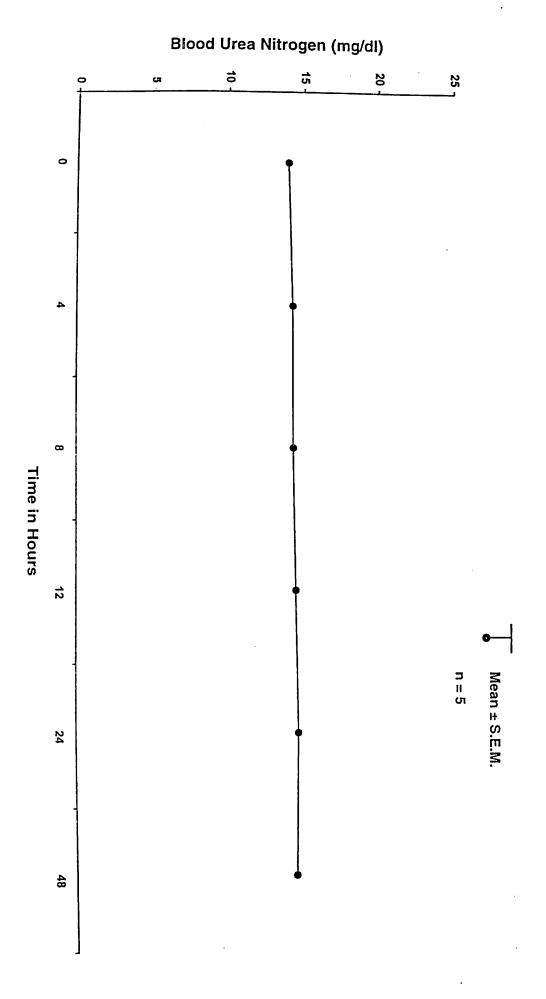
Table – 39 A

Showing analysis of data by single factor ANOVA
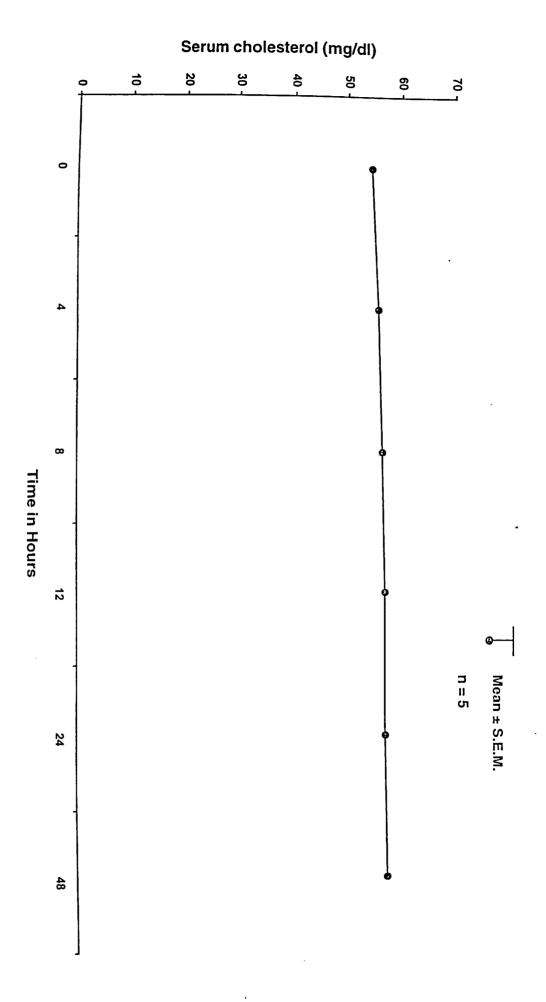
Sources of variation	D.F.	C.S.S.	M.S.	F
Between Days	5	378.12	75.62	6.98*
Error	24	259.87	10.82	

* $p < 0.05$.

❖❖❖❖❖

Fig. 18 : *Showing effect of daily s.c. injection of ivermectin at the therapeutic dose (0.66 mg/kg) for 14 days on various intervals of days in goats on serum glutamate oxaloacetate transaminase (SGOT) (IU/L)*

Fig. 17 : *Showing effect of daily s.c. injection of ivermectin at the therapeutic dose (0.66 mg/kg) for 14 days on various intervals of days in goats on Serum Glutanate Pyruvate Transaminase (SGPT) (IU/L)*

Fig. 16: *Showing effect of daily s.c. injection of ivermectin at the therapeutic dose (0.66 mg/kg) for 14 days on various intervals of days in goats on blood urea nitrogen (BUN in mg/dl)*

Fig. 15 : *Showing effect of daily s.c. injection of ivermectin at the therapeutic dose (0.66 mg/kg) for 14 days on various intervals of days in oats on serum cholesterol.*

Serum cholesterol (mg/dl)

Time in Hours

Mean ± S.E.M.

n = 5

Fig. 14 : *Showing effect of daily s.c. injection of ivermectin at the therapeutic dose (0.66 mg/kg) for 14 days on various intervals of days in goats on blood glucose level (mg/dl)*

Fig. 13 : *Showing effect of daily s.c. injection of ivermectin at the therapeutic dose (0.66 mg/kg) for 14 days on various intervals of days in goats on total plasma protein (g/dl)*

Fig. 12: *Showing effect of single s.c. injection of ivermectin at five time the therapeutic dose (3.3 mg/kg) at various time therapeutic dose (0.33 mg/kg) at various times intervals in goats on serum glutamic oxaloacetate transaminase (SGOT) (IU/L)*

SGOT (IU/L)

Time in Hours

Mean ± S.E.M.

n = 5

Fig. 11 : *Showing effect of single s.c. injection of ivermectin at the five time of therapeutic dose (3.3 mg/kg) at various time intervals in goats on Serum Glutanate Pyruvate Transaminase (SGPT) (IU/L)*

Fig. 10 : *Showing effect of single s.c. injection of ivermectin at five time the therapeutic dose (3.3 mg/kg) at various time intervals in goats on blood urea nitrogen (BUN in mg/dl)*

Fig. 9 : *Showing effect of single s.c. injection of ivermectin at five time the therapeutic dose (3.3 mg/kg) at various time intervals in goats on serum cholestrol (mg/dl)*

Serum cholesterol (mg/dl)

Time in Hours

Mean ± S.E.M.

n = 5

Fig. 8 : *Showing effect of single s.c. injection of ivermectin at five time the therapeutic dose (3.3 mg/kg) at various time intervals in goats on blood glucose level (mg/dl)*

Fig. 7 : *Showing effect of single s.c. injection of ivermectin at five time the therapeutic dose (3.3 mg/kg) at various time intervals in goats on total plasma protein (g/dl)*

Chapter - 5

Discussion

DISCUSSION

Ivermectin, an avermectin compound, is a potent anthelmintic and ectoparasiticide. It has excellent activity against broad spectrum of gastro-intestinal nematodes, lung worm and heart worm as well as systemic activity against several ectoparasites of domestic animals at extremely low dosage. Ivermectin is available for subcutaneous injection in cattle and pigs and for oral administration in horses and sheep. The drug persists longer in tissues after injection giving greater efficacy against parasites. The activity of the drug is upto 21 days in cattle after injection, but only about 7 days in sheep after oral administration. Ivermectin should not be used in lactating cattle because high levels are found in milk for long periods after administration.

Ivermectin is approved for use in majority of species like horse, cattle, swine, raindeer, bison etc. to treat various parasitic diseases. These species have not demonstrated any serious adverse effect after receiving recommended dose; but the dogs specially collies appear to be more sensitive to the toxic effects of ivermectin than other canine breeds. Most clinicians feel that ivermectin should not be used in collies or collie-mix breeds if alternative therapies are available.

Pharmacodynamic, pharmacokinetic and toxicity studies were carried out in all species of animals but very little work was done in goats, particularly studies on toxicity. Therefore, the present study was undertaken to know the toxicity of ivermectin in goats.

TOXICITY STUDIES :-

CLINICAL PARAMETERS

In the present study, acute toxicity effects of ivermectin at therapeutic dose (0.66 mg/kg) as well as at higher dose (5 times the therapeutic dose) of 3.3 mg/kg on different clinical parameters namely respiration rate, pulse rate, body temperature and ruminal movement were carried out after single s.c. injection at different time intervals viz., 0, 4, 8, 12, 24 and 48 h. Similarly, to study the long-term effect of the drug on different clinical parameters as noted above was done after s.c. injection of ivermectin (0.66 mg/kg daily for 14 days) at different intervals of day viz., 0. 1, 2, 4, 8 and 14th day.

The toxicity study at therapeutic dose revealed that there was no. significant change in respiration rate/min which varied from 23 ± 0.44 to 21.2 ± 0.37 from 0 to 24 hrs (Table 1 and 1A). The other parameter i.e. pulse rate, body temperature and ruminal movement also showed insignificant changes (Table 2, 2A, 3, 3A, 4 and 4A).

The acute toxicity study at higher dose i.e. 5 times the therapeutic dose (3.3 mg/kg s.c once only) revealed non-significant changes in respiration rate and the value varied from 20.8 ± 0.86 to 25.3 ± 1.44 from 0 to 48 h. Similar pattern on pulse rate, body temperature and ruminal movement which altered non-significantly (Table 15, 15A, 16, 16A, 17 and 17A) when ivermectin was given at higher dose of 3.3 mg/kg s.c.

The study on long-term effect of drug (0.66 mg/kg 14 days) also revealed that there was no significant change in respiration rate and the value varied from 21.6 ± 1.2 to 23.06 ± 0.74 from day 0 to 14. It was also observed that a similar pattern on pulse rate, body temperature and ruminal movement which altered non-significantly (Table 28, 28A, 29, 29A, 30, 30A) like in the case of acute effect of ivermectin when given at therapeutic dose (0.66 mg/kg) and higher dose (0.3.3 mg kg) once only by s.c.

It is well established that ivermectin acts by stimulating the release of gamma aminobutyric acid (GABA), a major inhibitory neurotransmitter. In mammals, GABA containing neurons and receptors are found in the central nervous system; while in arthropods and nematodes GABA is found primarily in the peripheral nervous system (neuromuscular function). This difference in location

of GABA receptor may be the reason for the large margin of safety of ivermectin-containing products in mammals (American Board of Veterinary Toxiology).

Ivermectin is well distributed in most tissues, but does not readily penetrate into CSF, thereby minimizing its toxicity. Collie-breed dogs may allow more ivermectin into the CNS than other breeds/species and hence the toxicity is seen more in this breed of dogs. In cattle and other mammals, the drug may not enter into CSF. In cattle, toxic effects generally do not appear until dosage of 30 times the therapeutic dose. Sheep showed symptoms of ataxia and depression at doses of 4 mg/kg. Swine showed symptoms of toxicosis (lethargy, ataxia, tremors, lateral recumbency and mydriasis) at dose of 30 mg/kg. In the present study, a maximum dose of 3.3 mg/kg s.c. was used in goat. Hence, it is expected that the drug may not cause any toxic symptom as shown by insignificant changes in all clinical parameters (respiration, pulse, body temperature and ruminal movement).

HAEMATOLOGICAL PARAMETERS

The present study was undertaken to explore the possible untoward effects of ivermectin on different haematological parameters viz., haemoglobin (Hb), total leucocyte count (TLC) and

differential leucocyte count (DLC) post single s.c. injection at therapeutic dose (0.66 mg/kg) at different time intervals of 0, 4, 8, 12, 24 and 48 h. Likewise, the toxicity study at higher dose (3.3 mg/kg) on the above noted haemotological parameters at different time intervals of 0, 4, 8, 12, 24 and 48 h was also done. Long term effects of ivermectin on different haematological parameters were noted after s.c. injection of ivermectin at therapeutic dose (0.66 mg/kg 14 days) at various intervals of days vig ; 0, 1, 2, 4, 8 and 14.

The toxicity study at therapeutic dose revealed that there was no significant change in haemoglobin and the value varied from 11.24 ± 0.11 to 11.8 ± 0.15 g/dl from 0 h 48 h (Table 5). Likewise, there was no significant effect on TLC (Table 6) and DLC (Table 7) of ivermectin at various time intervals of 0 h 48 h.

The toxicity study at higher dose (3.3 mg/kg, s.c.) revealed non significant effect on haemoglobin and the value varied from 9.46 ± 0.41 to 9.35 ± 0.35 mg/dl from 0 h 48 h (Table 18). Similarly, non-significant effect only was observed on TLC (Table 19) and DLC (Table 20). The study of long-term effect of ivermectin at therapeutic dose (0.66 mg kg for 14 days) revealed that there was no significant change in haemoglobin and the values varied from 9.36 ± 0.33 to 9.88 ± 0.24 g/dl from 0 to 14 days. (Table 31). It was also observed a similar pattern on TLC (Table 32) and TLC (Table 33), which altered non-

significantly. On the basis of haematological examination, it is concluded that ivermectin has no significant effect on different blood picture. The result is also in accordance with the finding of Remez et al. (1989) who also noted non-important changes in blood cell count in sheep following s.c. injection of ivermectin @ 0.2 mg/kg daily for the 14 days.

BIOCHEMICAL PARAMETERS

In the present toxicity study, untoward effects of ivermectin on different biochemical parameters namely total plasma protein, blood glucose, serum cholesterol, blood urea nitrogen (BUN), SGPT and SGOT were studied after its single s.c. injection of (0.66 mg/kg) at different time intervals *viz.*, 0, 4, 8, 12, 24 and 48 h. Likewise, the acute toxicity studies at higher dose (3.3 mg/kg s.c.) on various biochemical parameters at different time intervals viz, 0, 4, 8, 12, 24 and 48 h, were observed. Long-term effects of the drug on different biochemical parameters as noted above were studied after s.c. injection of (0.66 mg/kg for 14 days) at different intervals of days *viz.*, 0, 1, 2, 4, 8 and 14.

The toxicity study of ivermectin at therapeutic dose (066 mg/kg s.c. once only) revealed that there was no significant change in total plasma protein (gm/dl) and the value varied from 7.4 ± 0. 13 to

6.36 ± 0.07 from 0 to 48 hrs. (Table 8 and 8A) likewise, there was no significant effect on blood glucose level, serum cholesterol, SGPT and they differ non-significantly from 0 to 48 hr. (Table 9 & 9A, 10 & 10A, 12 & 12A)

The toxicity study at higher dose (3.3 mg/kg) revealed no significant effect on total plasma protein (gm/dl) and the value varied from 7.18 ± 0.14 to 7.30 0.10 from 0 to 48 hrs (Table 21 & 21A). It was also observed that there was no significant effect on blood glucose, serum cholesterol, BUN and SGPT, which altered non-significantly. (Table 22 & 22A, 23 & 23A, 24 & 24 A, 25 & 25A).

The study on long-term effect of the drug at therapeutic dose (0.66 mg/kg, s.c. for 14 days) also revealed that there was no significant change of total plasma protein and the values varied from 7.20 ± 0.01 to 7.38 ± 0.09 from day 0 to 14. (Table 34). It was also observed a similar pattern as noted above in the levels of blood glucose, serum cholesterol and SGPT, which altered non significantly (Table 35 & 35A, 36 & 36A, 38 & 38A).

A significant effect of ivermectin on serum glutamate oxaloacetate transaminase (SGOT) was observed in all the studies noted above. The toxicity study at therapeutic dose (0.66 mg/kg, s.c. once only) the value noted at 12 h (23.8 ± 0.66 IU/L), 24 h (25.8 ±

0.57IU/L) and 48 h (26.8 ± 0.58IU/L) are significantly higher from the value of 19.40 = 0.74 IU/L) 0 h (Table 13 & 13A). This indicates that ivermectin has a significant effect with increase in time. The normal value for SGOT in goat varied from 43-132 IU/L (Kaneko *et al.*, 1997). Though there is a significant effect of ivermectin on SGOT values obtained at different time intervals but the values are within the physiological limits.

The effect of ivermectin on SGOT during the study at higher dose (3.3 mg/kg s.c. once only) has been presented in Table 26 and 26A. The values for SGOT at 0 h (17.8 ± 1.3 IU/L), 4 h (18.45 ± 1.24 IU/L), 8 h (20.51 ± 1.12 IU/L), 12 h (22.33 ± 1.31 IU/L), 24 h (26.01 ± 1.10 IU/L) and 48 h (27.90 ± 0.93 IU/L) were obtained in the present study. All the above values noted on different hours differ with each other other but significantly differs at 48 h. This indicates the significant effect of ivermectin on SGOT.

In case of long-term effect study of ivermectin (0.66 mg kg s.c. once daily for 14 days), the value noted on day 8 (25.2 ± 1.20 IU/L) day 14 (28.8 ± 1.80 IU/L) differ significantly from the value noted on day 0 (18.42 ± 1.35 IU/L). This indicates that the drug has significant effect on SGOT value, which is more or less similar to the study of Locoenstein *et al.* (1996) and Hyatec *et al.* (1989). It has been

reported that SGOT increased 10,000 times in myocardial cells and 500 times in liver cells them the serum value in myocardial infarction and liver diseases (Vasudevan *et al.*, 1988).

The present toxicity studies on ivermectin reveal that the drug has no toxic effect on various clinical parameters (respiration, pulse, body temperature and ruminal movement), haematological parameters (haemoglobin, total leucocyte count, differential leucocyte count) and biochemical parameters (total plasma protein, blood glucose, serum cholesterol, blood urea nitrogen and SGPT); however, a slight increase in SGOT was noted, which is within physiological limit. Hence, the drug should be used carefully in animals predisposed with cardiac muscle and neurological disorders.

The absence of any adverse effect in host body indicates that ivermectin does not hamper the normal physiology of the host. The result were in concurrence with the finding of Islam *et al.* (1999) in donkey.

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Chapter - 6

Summary

SUMMARY

Assessment of adverse effects of ivermectin was carried out in five healthy adult goats of non-descript breed weighing between 16-22 kg with more or less similar age. For acute toxicity study, ivermectin was administered in each goat through s.c. route at therapeutic dose (0.66mg/kg) and at higher dose i.e. five times the therapeutic dose (3.3mg/kg) once only. Different clinical (respiration rate, pulse rate, body temperature, ruminal movement), haematological (Hb, TLC and DLC) and biochemical (blood glucose, serum cholesterol, total protein, BUN, SGPT and SGOT) parameters were estimated before treatment (0 h) and at 4, 8, 12, 24 & 48 h post injection. To study the long term effect, ivermectin was administered at therapeutic dose (0.66 mg/kg, s.c.) once daily for the 14 days and the above noted parameters were estimated on pretreatment (day 0) and on day 1, 2, 4, 8 and 14 of post treatment. The following results were noted.

A.   *Clinical parameters* :

In acute toxicity studies, values of respiration rate/min, pulse rate/min, body temperature (°F) and ruminal movement/5 min did not show any significant change at various time intervals (4, 8, 12, 24 and 48 h) from that of pretreatment (0 h) values after a single s.c.

injection of ivermectin at therapeutic dose (0.66 mg/kg) as well as at high dose (33 mg/kg). Similarly, the study on long term effect of therapeutic dose (0.66 mg/kg, s.c. once daily for 14 days) did not show any significant change on clinical parameters on various days of post treatment (day 1, 2, 4, 8 and 14) from that of pretreatment (day 0).

B. _Haematological parameters_ :

The acute toxicity study of ivermectin at therapeutic dose (0.66 mg/kg) and high dose (3.3 mg/kg) once only when given by s.c. route reveals non significant change in all haemotological parameters (Hb, TLC and DLC) at various time intervals of post treatment (4, 8, 12, 24, and 48 h) from that of pretreatment (0 h) values. Similarly, the study on long term effect on haemotological parameters by ivermectin at therapeutic dose (0.66 mg/kg, s.c. daily for 14 days) also revealed non-significant changes on different days of intervals (day 1, 2, 4, 8 and 14) from that of pretreatment (day 0). Thus, the study establishes that ivermectin even at high doses acutely and at therapeutic dose for a longer period may not affect the haemopoietic system.

C. _Biochemical parameters_ :

The acute toxicity study at therapeutic dose (0.66 mg/kg s.c.) and at high dose (3.3 mg/kg, s.c.) administered once only revealed non-significant changes on various bio-chemical parameters _viz.,_

blood glucose, serum cholesterol, total protein, BUN and SGPT at various time intervals of post treatment (4, 8, 12, 24, and 48 h) from that of pretreatment (0 h). Similar is the trend in case of long term effect of ivermectin at therapeutic dose (0.66 mg/kg, s.c. once daily for 14 days), which also showed non-significant changes on day 1, 2, 4, 8 and 14 post treatment from that of pretreatment (day 0) value of the above noted parameters.

The only parameter which showed significant changes is serum glutamic oxaloacetate transaminase (SGOT). The acute toxicity study at therapeutic dose (0.66 mg/kg s.c once only) on SGOT revealed that the value noted at 0 h (19.4 $\pm$ 0.74 IU/L) increased significantly at 12 h (23.8 $\pm$ 0.66 IU/L), which further increased at 24 h (25.8 $\pm$ 0.57 IU/L) and at 48 h (26.8 $\pm$ 0.58 IU/L). The acute toxicity study at high dose (5 times the therapeutic dose) also revealed that SGOT value increased significantly at 48 h as compared the pretreatment (0 h) value. The mean values of 17. 8 $\pm$ 1.34, 18.45 $\pm$ 1.24, 20.51 $\pm$ 1.12, 22.33 $\pm$ 1.3, 26.01 $\pm$ 1.10 and 27.90 $\pm$ 0.93 IU/L were noted at 0, 4, 8, 12, 24 and 48 h, respectively. Similar trend was observed on the long term effect of ivermectin at therapeutic dose (0.66 mg/kg, s.c. once daily for 14 days) revealed that the value noted for SGOT on day 8 (25.2 $\pm$ 1.20 IU/L) and day 14 (28.8 $\pm$1.80 IU/L) differed significantly from that of pretreatment (day 0) value (18.42 $\pm$

1.35 IU/L). The above data indicate the possible muscle damaging property of ivermectin, particularly on cardiac muscle.

It is noted that SGOT increase s 10,000 times in myocardial cell and 500 times in liver cells then the serum values in myocardial infarction and liver diseases, where as SGPT increases 3000 times in liver and 400 times more in heart in diseased condition (Vasudevan *et al.*, 1988). However, the significant increase in SGOT values noted in the present study after acute and long term administration of ivermectin is within the physiological limit. The normal value of SGOT in goat form 43 to 132 IU/L (Kaneko, 1997). Hence, the drug should be used carefully in animal predisposed with muscular disorders, specially in cardiac dysfunction.

By taking into account of various advantages and least toxicity as noted above, the drug can be used safely in the treatment or various ecto and endoparasites by s.c. route in animals, particularly goat. Further toxicity study should be conducted by administering for a longer period (chronic toxicity study) with special emphasis towards the effect of ivermectin on immune system, carcinogenic and teratogenic effects as well as effects on other vital organs.

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Appendix

APPENDIX – I

ESTIMATION OF HAEMATOLOGICAL PARAMETERS

(i) *Haemoglobin (Hb in gm/dl)*

The haemoglobin content of blood samples were estimated by Acid Haematin method using Hellige and sahli's haemoglobinometer as per procedure described by Schalm *et al.* (1975).

Principle - When blood is added to 0.1 N HCl, Hb is converted to brown coloured acid haematin and colour is matched with standards

Procedure :

1. 0.1 N HCl was added in the graduated tube upto the lowest mark (20% mark) using a Pasteur pipette.

2. Blood was drawn upto 20 µl mark in the Hb-pipette. Blood column was adjusted carefully without bubbles. Excess of blood on the sides on the sides of the pipette was wiped out using a dry piece of cotton.

3. Blood was transferred to the acid in the graduated tube and rinsed well. The reaction mixture was mixed properly and allowed to stand for at least 10 minutes.

4. The solution was diluted with distilled water by using a few drops at a time and mixed until the colour of the reaction mixture matches with the glass plate on the comparator against natural light.

5. The level of the fluid was noted at its lower meniscus and the reading corresponding to this level on the scale was recorded in gm/dl. This is the value of haemoglobin in grams per 100 ml of blood.

(ii) *Total Leucocyte Count (TLC)*

The total leucocyte count of blood was done using improved Neubauer chamber by the method as described by Boddie and Goe (1962).

Principle – Acid diluting fluid doesn't destroy WBCs. Stain added to diluting fluids makes nuclei visible in a counting chamber under the microscope.

Procedure : Blood was drawn in Throma WBC pipette having white bead upto 0.5 mark. If the blood has risen above the desired mark, pipette tip was touched with gauze and adjusted. Then, diluting fluid was drawn upto the mark 11. While drawing diluting fluid, the pipette was gently revolved. This kept bead in motion and prevented air

bubbles sticking to the bulb. The pipette was shaken for a few seconds while keeping finger or thumb on the tip of pipette. Rubber tubing was removed and pipette was held horizontally between thumb and finger. The content of pipette was mixed first, a few drops were discarded. The tip of the pipette was touched to haemocytometer. The fluid was allowed to flow by capillary action slowly and the counting chamber was filled without any air bubbles. Both the counting areas were charged and average of final report was taken. The cells were allowed to settle for 1-2 minutes. The counting chamber was placed under microscope and the cells in four large squares were counted with ruled area under low magnification (10 X).

Calculation –

Number of leucocytes / mm^3 = leucocytes in 4 WBC squares × 50.

(iii) *Differential Leucocyte Count (DLC)*

The differential leucocyte count of blood was done by the method as advocated by Boddie and Goe (1962).

Preparation of blood film – For differential leucocyte count, first a thin blood smear was prepared on a glass slide. For preparing blood film, a small drop of fresh blood was placed near one end of slide. The blood was spreaded evenly with a spreader slide at 30° angle. A good slide film should have smooth appearance, free from holes, straight

border and rainbow like appearance when seen against light. Then the blood film was air dried.

Staining – For staining few drops of undiluted Leishman's stain was added and allowed to act for 1 minute, then the stain was diluted with double amount of buffered distilled water and allowed the diluted stain to act for 5 minutes. The slide was washed gently with distilled water and air dried. A good stained slide had a pinkish tinge.

Examination of blood smear

The inspection of blood film is first done under low power magnification (10X). A portion of smear near the thin end referred as counting area was selected and switched to oil immersion lens (100X). Examination of blood smear was done thoroughly and at least 100 leucocytes were counted by battlement or zig-zag method. Counted cells were neutrophils, lymphocytes, monocyctes, eosinophils and basophils. Results were expressed in percentage.

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APPENDIX – II

ESTIMATION OF BIOCHEMICAL PARAMETERS

The estimation of biochemical parameters were done using commercially available kits from Nice and Span Diagnostics.

(i) Total plasma proteins

The estimation of blood proteins *i.e.* total protein and albumin were done by Biuret and Bromocresol method as advocated by Reinhold (1953).

Principle – The peptide linkages of amino acids in protein react with biuret reagent to form a violet coloured complex. This is measured colorimetrically at 530 nm.

Procedure

Three test tubes marked blank (B), standard (S) and test (T) were taken and 5 ml of biuret reagent was added to each of these. 0.1 ml of distilled water was added to the blank whereas 0.1 ml of serum was added to the test and 0.1 ml of protein standard was added to the standard. The content of tubes were mixed well and kept at room temperature for 30 minutes. Optical density was measured at 545 nm (green filter) against blank.

Calculation

Serum protein in gm % = $\dfrac{\text{O.D. of T}}{\text{O.D. of S}} \times 5$

(ii) Blood Sugar

The estimation of blood sugar was done by Folin and Wu method as described by Frakel *et al.* (1970).

Principle – The method is based on three stages –

(i)   Precipitation of blood proteins with copper tungstate.

(ii)   Reduction of cupric sulphate to cuprous oxide .

(iii)   Colorimetric measurement of the subsequent green colour produced on the addition of molybdate reagent to the cuprous oxide.

Procedure :

Test - 0.1 ml of whole blood was taken in a test tube containing 3.5 ml of distilled water, 0.2ml of sodium tungstate solution (10%) was added and mixed. Then, 0.2 ml of 2/3 N H_2SO_4 was added and mixed and allowed to react for 5 minutes and then centrifuged at 3000 rpm for 10 minutes. 2 ml of supernatant fluid was taken in a Folin and Wu tube marked T.

Blank – 2 ml of distilled water was taken in a Folin and Wu tube, marked B.

Standard – 1 ml of glucose working standard (0.1 mg / ml) and 1 ml distilled water was taken in a Folin and Wu tube marked S.

To each of the above tubes, 2 ml of alkaline copper tartarate reagent was added and mixed and placed in a boiling water bath exactly for 8 minutes, then cooled without shaking and added 2 ml of phosphomolybdic acid reagent and mixed. All the three tubes were allowed to stand for 5 minutes and diluted upto 12.5 ml mark with distilled water and mixed well. Readings were taken in colorimeter at 440 nm or by dark blue filter against blank set at zero.

Calculation – mg glucose in 100 ml blood $= \dfrac{\text{O.D. of T}}{\text{O.D. of S}} \times 200$

(iii) Blood Cholesterol

The estimation of blood cholesterol was done by Ferric Chloride method as described by Wootton (1964)

Principle – Cholesterol reacts with Ferric chloride in the presence of acetic acid and sulphuric acid. The red colour thus produced is measured colorimetrically.

Procedure : First, a working reagent was prepared by diluting 0.5 ml of ferric chloride to 50 ml with aldehyde free acetic acid. 9.9 ml of this

working reagent was pipetted into a centrifuge tube and 0.1 ml of serum was added, mixed and allowed to stand for 15 minutes and then centrifuged. Thus, a protein free solution was obtained. Three test tubes marked as blank (B), standard (S) and test (T) were taken. 5 ml and 4.9 ml of working reagent was added to B and S, respectively. 5 ml of protein free solution was added to the test. 0.1 ml of cholesterol standard was added to S. Thereafter, 3 ml of concentrated sulphuric acid (36 N) was added to each test tube, mixed and allowed to stand for 30 minutes in the dark. Optical density was measured at 560 nm (yellow green filter) against distilled water set at zero.

Calculation

$$\text{Blood cholesterol in mg per 100 ml of blood} = \frac{T - B}{S - B} \times 200$$

(iv) Blood Urea Nitrogen (BUN)

The blood urea nitrogen was estimated by Diacetyl Monoxime method as described by Wootton (1964).

Principle – Urea reacts with diacetylmonoxime in the presence of an activator to form a pink coloured derivative. This is measured colorimetrically.

Procedure : First, dilution of serum and urea standard (1 : 20) was done with distilled water. Three test tubes marked as blank (B), standard (S) and test (T) were taken and then 2.0 ml of acid reagent was added to all the test tubes. 0.2 ml of distilled water was added in the blank. 0.2 ml of diluted standard and diluted serum were added to standard and test, respectively. Then, 2 ml of colour reagent (diacetylmonoxime 2% solution in 2% acetic acid) was added to each of the tubes and mixed properly.

After mixing, the tubes were placed in a boiling water both exactly for 10 minutes and then cooled. Optical density was recorded at 540 nm (green filter) against blank.

Calculation

$$\text{mg of BUN per 100 ml of blood} = \frac{\text{O.D. of T}}{\text{O.D. of S}} \times 40 \times 0.467$$

(v)   **Serum Glutamate Pyruvate Transaminase (SGPT) or Alanine Transaminase (ALT) :**

The estimation of SGPT was done by the 2,4- DNPH method as described by Reitman and Frankel (1957).

Principle

Alanine + α - ketoglutaric acid $\xrightarrow{\text{SGPT}}$ Pyruvic acid + gludamic acid

Pyruvic acid so formed is treated with 2,4 – DNPH and the brown colour produced is measured colourimetrically at 505 nm.

Procedure : Preparation of standard graph for SGPT -

Pipetted in the tubes labelled as below :-

	1	2	3	4	5
Buffered Alanine (ml)	0.5	0.45	0.4	0.35	0.3
Pyruvate standard (ml)	-	0.05	0.1	0.15	0.2
Distilled water (ml)	0.1	0.1	0.1	0.1	0.1
DNPH reagent (ml)	0.5	0.5	0.5	0.5	0.5
Mixed well and allowed to stand at room temperature for 20 min.					
0.4 N NaOH (ml)	5.0	5.0	5.0	5.0	5.0
Enzyme activity IU/L	0.0	13.4	27.4	46.6	72.0

The contents of the tubes were mixed and kept at room temperature for 10 minutes. Optical densities were measured at 505 nm (green filter) against purified water. A graph was plotted by drawing O.D. on y-axis and IU L on x-axis.

Test - 0.25 ml of buffered alanine was taken in a test tube marked T and incubated at 37^{0}C for 5 minutes. Then, 0.05 ml of serum was placed in the tube, mixed and again incubated at 37^{0}C for 30 minutes. Thereafter, 0.25 ml of DNPH colour reagent was added to the tube,

mixed and allowed to stand for 20 minutes at room temperature. Finally, 2.5 ml of 0.4 N NaoH was added, mixed and allowed to stand for 10 minutes. Optical density was measured at 505 nm against purified water.

Calculation – O.D. of T was marked on y - axis of the standard curve and it was extrapolated to the corresponding enzyme activity on x - axis.

(vi) Serum Glutamate Oxaloacetate Transaminase (SGOT) or Aspartate Transaminase (AST) :

.The estimation of SGOT was done by 2, 4-DNPH method as described by Reitman and Frankel (1957).

Principle

Aspartic acid + α ketoglutaric acid $\underset{}{\overset{SGOT}{\rightleftharpoons}}$ Oxaloacetic acid+ glutamic acid.

Oxaloacetic acid so formed is coupled with 2, 4 - dinitrophenylhydrazine (2, 4 - DNPH) to give the corresponding hydrazone, which gives brown colour in alkaline medium and this is measured colorimetrically.

Procedure : Preparation of standard graph for SGOT :

Pipetted in the tubes labelled as below : -

	1	2	3	4	5
Buffered Alanine (ml)	0.5	0.45	0.4	0.35	0.3
Pyruvate standard (ml)	-	0.05	0.1	0.15	0.2
Distilled water (ml)	0.1	0.1	0.1	0.1	0.1
DNPH reagent (ml)	0.5	0.5	0.5	0.5	0.5
Mixed well and allowed to stand at room temperature for 20 min.					
0.4 N NaOH (ml)	5	5	5	5	5
Enzyme activity (IU/L)	0.0	11.5	29.3	54.7	91.2

The content of the tubes were mixed well and allowed to stand at room temperature for 10 minutes. Optical densities of all the five tubes were measured against distilled water at 505 nm (green filter). A graph was plotted by drawing O.D. on y - axis and IU/L on x-axis.

Test - 0.25 ml of buffered aspartate was taken in a test tube marked T and incubated at 37°C for 5 minutes. Then, 0.05 ml of serum was added to the tube, mixed and again incubated at 37°C for an hour. 0.25 ml of DNPH colour reagent was added to the tube, mixed and

allowed to stand for 20 minutes at room temperature. Finally, 2.5 ml of 0.4 N NaoH was added to T and mixed and allowed to stand for 10 minutes. Optical density of T was recorded against distilled water at 505 nm (green filter) on a colourimeter.

Calculation

O.D. of T was marked on the y - axis of the standard graph and it was extrapolated to the corresponding enzyme activity on x-axis.

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