UNIVERSITI PUTRA MALAYSIA

THE EFFECTS OF CURCUMA LONGA (TURMERIC) AND LEVAMISOLE ON IN-VITRO SURVIVAL RATE OF STRONGYLES IN SHEEP

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THE EFFECTS OF *CURCUMA LONGA* (TURMERIC) AND LEVAMISOLE ON IN-VITRO SURVIVAL RATE OF STRONGYLES IN SHEEP

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It is hereby certified that we have read this project paper entitled "The Effects of *Curcuma Longa* (Turmeric) and Levamisole on In-Vitro Survival Rate of Strongyles in Sheep" by Norisal Binti Nasai and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4999- Project.

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DEDICATIONS

I dedicate this thesis with love and appreciation to:

My parents and siblings

SALOMA HUSSIN and SYD ROSELAN SYD OTHMAN

HAFIQ HAFIFI, SYARIFAH SYAZWANIE and SYARIFAH SYAZWAHIDA

My friends and FYP mate

HAFIZAH HAMID

'IZZATY HALIL

CHAI AITING

SYAIRAH RAMLI

My Coursemates

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ABSTRAK

Abstrak daripada kertas kerja projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999-Projek

KESAN *CURCUMA LONGA* (TUMERIC) DAN LEVAMISOLE KE ATAS KADAR SURVIVAL IN-VITRO STRONGYLE DALAM BEBIRI

Oleh:

Norisal Binti Nasai

2015

Supervisor: Dr. Murugaiyah Marimuthu

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dan

Prof. Madya Dr. Shaikh Mohamed Amin Babje

Cacing usus merupakan satu masalah global dalam pengeluaran ruminan kecil. Kebanyakan parasit akan menjadi imun terhadap kimia anti-parasit yang digunakan secara beterusan dalam jangka masa yang panjang. Kajian ini menilai kegunaan *Curcuma longa* sebagai racun nematod secara biologi di dalam rawatan parasit sistem

usus terutamanya strongyle dalam bebiri. Tujuan kajian ini dijalankan adalah untuk membandingkan tahap keberkesanan *Curcuma longa* dan Levamisole ke atas tahap ketahanan strongyle dalam bebiri dan untuk menentukan kepekatan paling efektif bagi ekstrak *Curcuma longa* aktiviti larvacidal terhadap larva strongyle tahap ketiga (L3) secara in-vitro. Dalam kajian ini, L3 dikulturkan daripada tinja bebiri yang dijangkiti semulajadi dan L3 diambil dari kultur tinja selepas 7 hari. Ekstrak methanol *Curcuma longa* disediakan dalam 3 dos yang berbeza iaitu 50, 100 dan 200 mg/ml dan keberkesanan ekstrak diuji ke atas L3. Levamisole pada dos 1.5 mg/ml dan 3.0 mg/ml dijadikan sebagai kawalan positif. Ekstrak *Curcuma longa* menunjukan aktiviti anti-parasit bergantung kepada tahap kepekatan dengan 78% cacing mati selepas 24 jam didedahkan kepada ekstrak pada kepekatan yang tertinggi iaitu 200 mg/ml. Kadar kematian cacing adalah 72% selepas 24 jam didedahkan kepada Levamisole, 1.5 mg/ml. Ekstrak kunyit, 200 mg/ml mempunyai kesan yang sama ke atas kadar kematian cacing jika dibandingkan dengan Levamisole, 1.5mg/ml ($p>0.05$). Tiada kematian cacing diperhatikan di dalam air suling selepas 24 jam kajian dijalankan. Kajian selanjutnya dicadangkan bagi menentukan aktiviti anti-parasit ekstrak *Curcuma longa* pada dos yang lagi tinggi.

Kata Kunci: *Curcuma longa*, Strongyle, L3, Levamisole.

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999-Project

THE EFFECTS OF CURCUMA LONGA (TURMERIC) AND LEVAMISOLE ON IN-VITRO SURVIVAL RATE OF STRONGYLES IN SHEEP

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Gastrointestinal helminth is a global problem in small ruminant production. Most of the parasites were found to develop resistance since chemical anthelminths have been used for a long period of time. The present study evaluated *Curcuma longa* as a biological nematicide in the treatment of the gastrointestinal helminth especially for strongyles in sheep. The objectives of the study were to compare the efficacy of

Curcuma longa and Levamisole on the survival rate of the strongyles in sheep and to determine the most effective concentration of the *Curcuma longa* extract for larvacidal activity against strongyle third stage larvae (L3) in in-vitro condition. L3 of the strongyles were cultured from the feces of naturally infected sheep and after 7 days, the L3 were harvested from the fecal culture. Methanolic extract of *Curcuma longa* were prepared into three different concentrations: 50, 100 and 200mg/ml, and the efficacy of the extract on the L3 was tested. Levamisole at concentration 1.5 mg/ml and 3.0 mg/ml were used as the positive control. The *Curcuma longa* extract showed anthelmintic activity in a dose dependent manner with 78% worms died within 24-hour exposure at the highest dose rate of 200 mg/ml. The mortality rate was compared with Levamisole 1.5mg/ml where 72% worms died after 24-hour exposure. Therefore, there is no significance difference between the effects of using Curcuma longa, 200mg/ml and Levamisole, 1.5mg/ml ($p>0.05$). There was no mortality of worms in distilled water for a 24-hours observation. Further research could be done to determine the antihelmintic activity of the *Curcuma longa* extract at higher doses.

KEYWORDS: *Curcuma longa*, strongyles, L3, Levamisole.

Chapter 1

INTRODUCTION

1.1 Background

The gastrointestinal helminth is one of the most significant factors causing retarded growth in ruminants by sharing the nutrients with their host. It is also one of the abiding threats to the livestock population of the world (Ullah *et al*., 2013). Treatment of the disease caused by these parasites is difficult as the parasites tend to develop resistance against the chemical drugs available commercially in the market. Thus there is an increasing need for natural anthelmintic treatment. Organic compounds from terrestrial and marine organisms have been used extensively in the treatment of many diseases and serve as compounds of interest both in their natural form and as templates for synthetic modification (Hrckova *et al*., 2013). Turmeric is said to have anti-inflammatory, hypocholestraemic, choleratic, antimicrobial, insect repellent, antirheumtaic, antifibrotic, antivenomous, antiviral, antidiabetic, antihepatotoxic as well as anti-cancerous properties in day to day used domestically (Velayudhan *et al*., 2012). It's used as an anthelmintic in goat has not been studied extensively.

1.2 Justification

Gastrointestinal helminth is a global problem in the small ruminant production. Most of the parasites would develop resistance if chemical anti-helminth drugs are used for a long period of time. This study would evaluate the significant success rate of using

Curcuma longa as the treatment of the gastrointestinal heminths especially for strongyles in sheep. This will help to give choice for the farmer either to choose between chemical treatments or natural remedies in their farm as sometimes using the chemical treatment would have residual effects on the sheep.

1.3 Study Objectives

a)) To compare the efficacy of *Curcuma longa* and Levamisole on the survival rate of the gastrointestinal helminths in sheep.

b) To determine the most effective concentration of the *Curcuma longa* extract for larvacidal activity against strongyles third stage larvae (L3) in in-vitro condition.

1.4 Study Hypothesis

We hypothesized that there is an effect of *Curcuma longa* on the strongyles in sheep.

Chapter 2

LITERATURE REVIEW

2.1. Strongyles in Small Ruminants

Generally strongyles would include *Haemochus sp., Tricostrongylus sp., Oesophagostomum sp*., and *Ostertagia sp.* and they are most common worms found in grazing ruminants with worldwide distribution (Ballweber, 2001). Among the helminth parasites of livestock, the gastrointestinal nematodes (GIN) have arguably the greatest overall impact (Sutherland and Scott, 2010). The species that affect animals may differ according to the animal species and regions. In sheep or small ruminants, *Haemonchus contortus* is the most important nematode that can cause Parasitic Gastroenteritis (PGE) problem other than *Tricostrongylus sp.* and *Oesophagostomum sp* and usually mixed infection will cause disease in animals. The majority of sheep and goats were infected by two and more parasite types with some animals showing pure infection (Zeryehun, 2012). These infections cause losses in production due to morbidity and in some cases mortality (Hutchinson, 2009). Ruminants acquire infection by ingestion of infective L3 and depending on species larvae may migrate into gastric glands, and then return to the lumen where they reach adult stage in 2 to 3 weeks (Ballweber, 2001).

Different species of nematodes can be found at different parts of the gastrointestinal system, for example, *Haemonchus contortus* is the largest of the abomasal parasites other than *Tricostrongylus sp. Tricostrongylus sp.* is the smallest abomasal and small intestine nematodes, and therefore it is relatively easily overlooked.

Oesophagostomum sp. is an example of nematode that can be found in large intestine. *Haemonchus contortus* and *Trichostrongylus sp*. are reported to be the most prevalent and highly pathogenic parasites in livestock, particularly in small ruminants (Tan *et al*., 2014).

2.2. *Curcuma longa* (Turmeric)

Under family of Zingiberaceae, *Curcuma longa* is the species that has many taxa of economic, medicinal, ornamental and cultural importance (Velayudan *et al*., 2011). It is a perennial herb that has oblong, pointed leaves and oval rhizomes which are often branched and brownish-yellow in colour. It has the characteristic odour and slightly bitter in taste. Turmeric is an English name for the herb and the plant is known by different vernacular names, for example in India it is being known as Haldi, pasupu in Telugu, kunyit in Malay, ukon in Japanese, halud in Benggali and aarishnina in Kannada (Ahmad *et al*, 2010). The main pigment and active constituent of turmeric is 1,7-bis-(4-hydroxy-3-methoxy-phenyl)-hepta-1,6-diene-3, 5-dione (Stankovic, 2004) or also known as curcumin, an orange-yellow volatile oil (Watson and Preedy, 2008).

Curcuma longa has a phenolic compound known as curcuminoid that contributes mostly to its biological activities. Main chemical constituents of turmeric would include the essential oils, polyphenols, turmeric oil, veleric acid, free acid (Coporioc acid), sugar, protein, fat, mineral oils, carbohydrates and resins. The aroma of turmeric is due to its volatile oil and its bright yellow colour is given out by the phenolic compound or curcuminoids (Watson and Preedy, 2008). Turmeric possess various biological activities

like antioxidant activity, antipsoriatic, antiplatelet, anti-haemolytic , chemopreventive action, antiapoptotic, radioprotective, antiatherogenic, antiinflammatory, antifungal, anti-viral, insecticidal and nematicidal, antidiabetic, antiageing and lipid lowering, neuroprotective (Alzheimer's disease), antiallergic and immunological system activating properties, antibacterial, wound healing and miscellaneous activities (Watson and Preedy, 2008; Ahmad *et al.,* 2010). Turmeric has specific therapeutic effects depending on the size of the animals, for example in canine the typical dosage of curcumin needed is 20-250 mg for three times a day and equine dosage range between 1200-2400 mg per day due to its big size. Due to its inert properties, there is no toxicity reported both in animals and humans, therefore, it is fairly safe to be used in daily life.

2.3. The Methanolic Extraction Method

Curcumin is the product obtained by solvent extraction of turmeric. The JECFA specifications monograph for Curcumin (FNP 52 Add. 9, 2001) lists acetone, methanol, ethanol, and isopropanol as suitable solvents (Stankovic, 2004). The process of extraction requires the turmeric to be ground into powder form before the curcumin can be extracted. In order to get turmeric in powder from, first it need to be completely dried at room temperature. Stankovic (2004) reported that methanol can be a good solvent that aids in the purification of curcumin in turmeric. According to JEFCA (2003), in methanol extract, the residual solvents are not more than 50 mg/kg. Curcumin has high solubility with methanol which means that it is solute when being used together that makes it difficult to separate both solvent and solute (Popuri and Pagala, 2013).

2.4. Levamisole Usage in Endoparasite Management

Levamisole is a nicotinic agonist anthelmintic drug together with butamisole, pyrantel, methyridine and others and it is under the class of imidazothiazoles. Levamisole, pyrantel and morantel are agonists at nicotinic acetylcholine receptors of nematode muscle and cause spastic paralysis (Martin, 1997). According to Williamson (2013), levamisole kills worms by depolarizing nicotinic neuromuscular junction and it has narrow therapeutic index and can cause toxicity that mimic organophosphate toxicity symptoms like hypersalivation, trembling, urination, defecation, collapse and death. Toxicity can occur when the drugs were overdosed over 30%. In sheep, it has 3 day withdrawal period for meat production and zero day milk withdrawal and the oral route is considered to be much safer compared to injectable route.

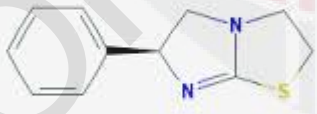

Figure 1. Chemical structure of Levamisole

Levamisole typically is effective against roundworms as well as hookworms but not in whipworms (Martin and Robertson, 2007). According to Martin and Robertson, (2007), resistance to cholinergic agonists may be produced by four general mechanisms:

(1) Changes in drug translocation (e.g. increased metabolism or excretion of the drug)

(2) Changes in receptor numbers (e.g. reduced number of receptors)

(3) Receptor modification (e.g. change in receptor binding sites due to amino acid substitution)

(4) Post-receptor modification (e.g. change in the downstream pathway to contraction)

2.5. The Effects of Turmeric and Levamisole as Anthelmintic

Turmeric extract showed good growth suppression on earthworm muscle cells when compared to ginger and garlic extracts (Vidya *et al.,* 2012) and *Curcuma longa* rhizome extracts also bearing a potential anthelmintic property when compared to standard drug concentration (Singh *et al.,* 2011). Turmeric extracts inhibited egg hatching and thus indicated their ovicidal effects and has been shown to inhibit ulcer formation caused by helminths in the form of stress, release of alcohol, endomethacin, pyloric ligation which significantly increased gastric wall mucus in animals subjected to the gastrointestinal insult (Ullah *et al.,* 2013). The turmeric killed helminths by interfering the worm energy generation and curcuminoid were the responsible natural phenols that showed the anthelmintic activity.

Levamisole hydrochloride is the main content of the levamisole drugs. As the cholinergic anthelmintic, it acts on nematode nicotinic acetylcholine receptors located on somatic muscle cells and causing them to be paralyzed. The effect of Levamisole on the worm is almost similar to the effect of the turmeric extract.

Chapter 3

MATERIALS AND METHODS

3.1 Materials Preparation

Plant materials: *Curcuma longa* (turmeric) rhizomes were collected from the field in University Putra Malaysia (UPM), Serdang area. These rhizomes were washed thoroughly in water, cut into smaller pieces and left to be dried at room temperature for 4 days. After that, the dried turmeric were ground to the powder form and stored at room temperature.

3.2 Fecal Culture and Harvesting L3

Fecal samples taken from the naturally infected sheep were crushed and placed in a glass jar that already been washed with distilled water. The moisture of the fecal in glass jar was maintained everyday by putting a few drops of distilled water on the feces for 7 days. The feces were not over-moistened to avoid fungal growth. After 7 days, the L3 were harvested from the culture. For the harvesting process, warm distilled water was used by filling it in the glass jar with the feces. After that, the petri dish was placed on top of the glass jar and turned it over before filling in the remaining warm distilled water into the petri dish. After 30 minutes, the L3 were observed under a dissecting microscope. The L3 were harvested into the glass bottle that already been washed with distilled water and kept it in a cold temperature as to maintain their dormant state.

3.3 Identification of Worm

One hundred third stage larvae, L3, were taken randomly from the harvested larvae. The L3 were put on the glass slide and 1 drop of Lugol's iodine was added before cover slip was put onto it. The samples then were identified under a light microscope.

3.4 *Curcuma longa* (Turmeric) Methanolic Extract

The extraction requires the turmeric rhizomes to be dried and ground into powder by using the grinder. The powder was air dried to remove the moisture that was still present in the ground powder. About 150 g of fine powder would be obtained from 1.5 kg fresh turmeric rhizomes. After that, 100 g turmeric powder were weighed accurately and then placed in 1litre glass jar before 1 litre of 99.8% methanol were added into the glass jar. Then, the glass jar was shaken manually for 20 minutes. The mixture was kept at room temperature for 3 days. After 3 days, the mixture was filtered with filter paper No. 1 Whattman England by using a vacuum pump. After that, the filtrate were poured into boiling flask and evaporated under vacuum using Rotary Vacuum Evaporator at 40^0C and speed 60 rpm to remove the methanol from the filtrate. The concentrate was left at room temperature for 24 hours to make sure the leftover methanol evaporated from the concentrate. Next, the concentrate was then further diluted with distilled water at ratio 200:1 to prepare the extract stock solution 200 mg/ml. The rest of the extract concentration was prepared by diluting the stock solution with distilled water.

3.5 Preparation of Levamisole

1 ml Levamisole with concentration of 32 mg/ml was diluted with 0.05 ml and 0.09 ml of distilled water to get concentration 1.5mg/ml and 3.0mg/ml respectively.

3.6 Treatment and Motility Assessment

Fifty active L3 were counted under a dissecting microscope in a petri dish. About 8.0 ml distilled water was added to maintain the L3. The L3 were treated with 1.0 ml *Curcuma longa* extract at 3 concentrations; 200 mg/ml, 100 mg/ml and 50 mg/ml. 1.0 ml Levamisole at concentration 1.5 mg/ml and 3 mg/ml were used as positive controls and 1.0 ml distilled water was used as negative control. Table 1 illustrated the treatments strategy of the Curcuma longa and Levamisole for their anthelmintic activity. Petri dishes were shakened manually for 1 minute after starting the treatment and were kept at room temperature. Each petri dish was examined every 2, 4, 6 and 24 hours post treatment. Four replicates were done for each treatment. For every 2 hours, the motility of the L3 was checked and recorded as motile and non-motile or dead L3. The dead L3 were confirmed by observing for the absence of L3 motility for up to 10 seconds. The mortality index formula was used to determine the rate of L3 mortality.

$$\text{Mortality Index} = \frac{No\ of\ dead\ L3}{Total\ number\ of\ L3\ in\ petri\ dish} \times 100\%$$

Table 1: Treatments strategy of the *Curcuma longa* and Levamisole for their anthelmintic activity

Treatment	Formulation	Dosage, mg/ml
Petri dish 1, 2 and 3	*Curcuma longa*	50, 100, 200
Petri Dish 4 and 5	Levamisole	1.5 and 3.0
Petri Dish 6	Distilled water	

3.7 Statistical analysis

The statistical analysis was performed using the SPSS version 20.0 statistical software. One-way ANOVA was used for normally distributed data and $p<0.05$ was considered to be significant. Independent sample T-test was used for comparison of data in two independent or unrelated groups which were turmeric extract highest concentration and Levamisole.

Chapter 4

RESULTS

Before the experiment was conducted, identification was done on 100 strongyles that were harvested from the fecal culture. From that, it was found that the harvested strongyles comprised of 97% *Haemonchus contortus* and 3% *Trichostrongylus sp*.

4.1 Different Concentrations of Turmeric

The results showed there were different effects exhibited by different concentrations of turmeric on the strongyle mortality ($p<0.05$). There was also increased number of dead strongyles over time. The turmeric concentration exhibited highest anthelmintic activity in a dose dependent and the maximum effect was at the highest dose of turmeric extract, 200 mg/ml where 78% worms died 24-hour post-exposure (Figure 2). The mean mortality of strongyles showed that it increased with increased of turmeric extract concentration (Figure 3). Mortality of strongyles was compared to the Levamisole as the reference drug or positive control at concentration of 1.5 mg/ml at 24-hour post-exposure and 3.0 mg/ml at 2-hour post-exposure. For levamisole, 1.5 mg/ml, 72% strongyles mortality observed till 24-hour post-exposure and for levamisole, 3.0 mg/ml, 100% strongyles died at 2-hour post exposure. Then, for negative control, there was no mortality of strongyles observed in distilled water up to 24-hours post-exposure.

4.2 Comparison Effects of Turmeric at Highest Concentrations and Levamisole 1.5mg/ml

The effects of turmeric extract were compared at highest dosage of 200 mg/ml with Levamisole at 1.5 mg/ml. Result showed that there was no significant difference between the effect of turmeric extract, 200 mg/ml and Levamisole, 1.5 mg/ml on the strongyles mortality over 24-hour post exposure ($p<0.05$). Levamisole at concentration 1.5 mg/ml, killed 72% of the worms at 24-hour post-exposure (Figure 4).

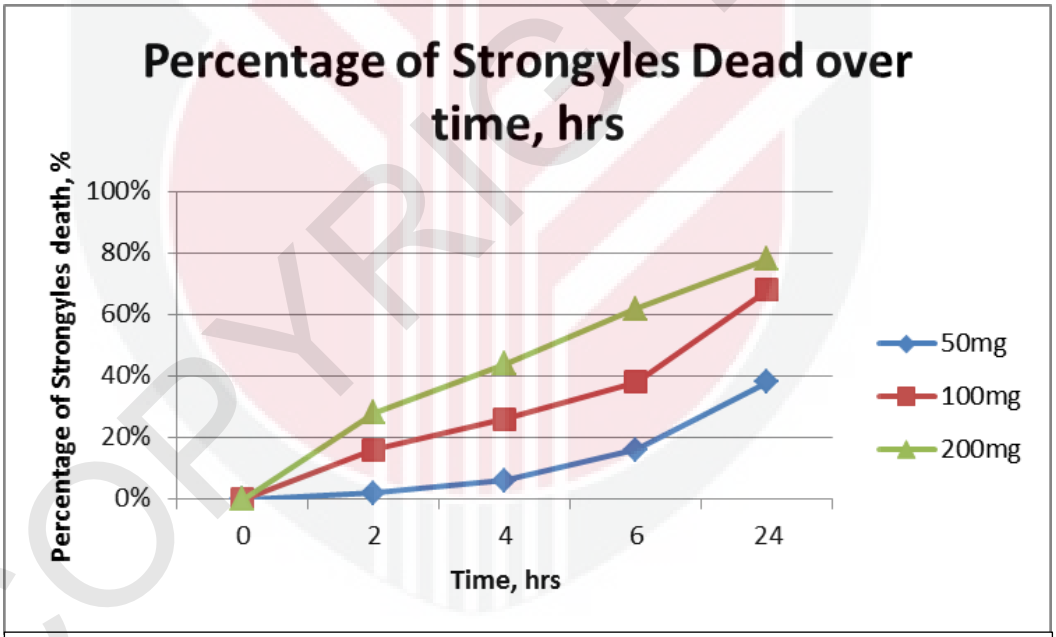

Figure 2: Effects of turmeric extraction at different concentration and the highest concentration showed highest anthelmintic effect where 78% worms died 24-hours post-exposure compared to other concentrations.

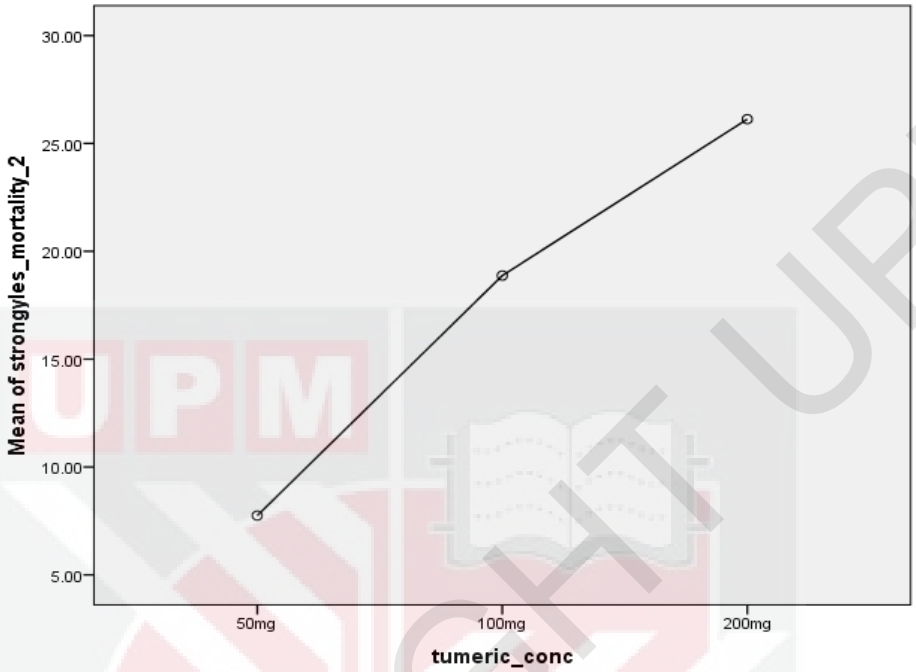

Figure 3: Mean mortality of strongyles that increased over the increased of turmeric extract concentration.

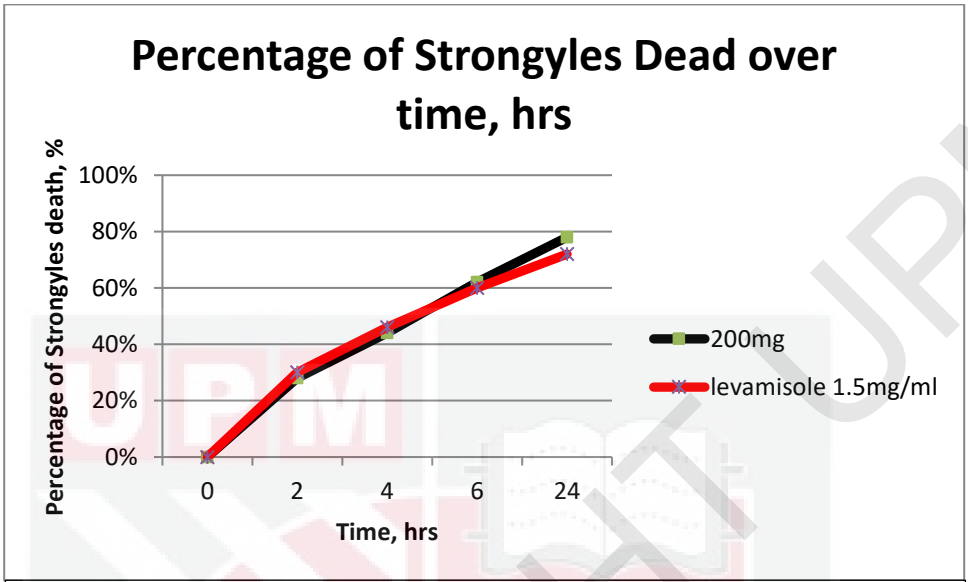

Figure 4: Effects of turmeric extraction at 200mg/ml where 78% worms died and Levamisole, 1.5mg/ml 72% worms died 24-hours post-exposure.

Chapter 5

DISCUSSION

Turmeric hydro-alcoholic extracts showed a remarkable anthelmintic potential against intestinal parasitism (Singh *et al.*, 2011). According to Kulkarni *et al.* (2012), maximum concentration of curcuminoids was obtained in methanol extract in the form of dark black orange colour compared to other extraction solvent like acetone, chloroform and ethyl acetate. This means that, methanol is a good solvent to be used to extract curcumin content compared to any other solvents.

Herbal extracts exhibited anthelmintic activity in a time and dose dependent manner as compared to negative control and effects of *Curcuma longa* as an anthelmintic revealed that some parts of cucurbits possess anthelmintic properties due to secondary metabolite cucurbitacin contents (Ullah *et al*., 2013). Previous studies reported that worms exposed to turmeric extract paralyzed and died in time and dose dependent manner. The effect of turmeric extract on earthworm muscle cells was found to be dosage dependent (Vidya *et al*., 2012). As in this experiment, it proved that with the increase in time and dose, the number of strongyles died also increased. This could be due to the presence of more cucurminoid content in the highest dosage exposed to the worms that caused increase in worm's mortality.

With the increasing problem of anthelmintic resistant problem in small ruminants especially sheep, there is increasing demand or need to find alternative treatments for the helminthiasis problems in ruminants. According to Schoenian (2012),

Haemonchus contortus is the internal parasite that causes major problem in warm and moist climatic area and secondary to *Haemonchus contortus* is *Trichostrongylus sp.* In this project, 100 strongyles were identified before being introduced to the treatment and the result showed from 100 worms 97 of them were *Haemonchus contortus* and just 3 of them were *Trichostrongylus sp.* For this experiment, the fecal samples were taken from a sheep farm that their egg per gram (epg) was above 250. The highest epg recorded was from a sheep with more than 100 000 epg. In this farm, the helminths were resistant to almost all anthelmintics that usually used like Albendazole, Closantel and Ivermectin as fecal egg reduction test was done on 40 sheep. From the test, it showed that the sheep's helminth were only sensitive to Levamisole.

In this study, two different concentrations of Levamisole were used: 1.5 mg/ml where 72% worms were dead 24-hour post exposure and 3.0 mg/ml where 100% worms were dead 2-hour post exposure. There is no significance difference between the effects of Levamisole and turmeric extract at 200 mg/ml on the number of strongyles died within 24-hour post treatment. Levamisole affect the worms by depolarizing nicotinic muscular junction and cause the worms to paralyse. While for turmeric, the curcuminoid is the main component that causes the worm's mortality by suppressing the worms muscle cell. A previous study by Vidya *et al.* (2012) showed that turmeric extract showed good growth suppression with respect to the cell count and cell viability. The test was done on earthworm muscle cells but since nematode or helminthes are only one level lower than earthworm, therefore, the project was valid to be considered that turmeric extract have similar effect on nematode as earthworms. Curcumin is the natural

polyphenol that is responsible for the anti-proliferative activity on dividing cells causing the muscle growth suppression of earthworms.

Chapter 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Turmeric concentration at 200mg/ml showed the highest anthelmintic properties with 78% L3 died within 24-hour experiment and was to the anthelmintic activity of Levamisole 1.5 mg/ml**.** Therefore it can be concluded that turmeric is an appropriate herb that can be used by resource poor farmers as an alternative anthelmintic as the plant is commonly used and easily available. Further research should be to obtain the most effective concentration of turmeric extract that would exhibit a better larvacidal activity of strongyles.

6.2 Recommendations

As for the recommendations for future study are,

- Further research at turmeric extract concentration of more than 200 mg/ml and to obtain the most effective concentration of turmeric extract that expressed lethal effect on the srongyles larvae.
- In-vitro studies of turmeric using different extraction techniques like aqueous extraction or by using different solvents for example acetone or ethyl ether.
- Study more extensively on the active ingredients produced in different extractions of turmeric to know which components that exhibit anthelmintic effect on worms.

- In vivo studies on animal model which could exhibit any inhibitory mechanism in animals and to study on the different modes of administration that can appropriately or suitable to be used by the farmers.
- Study the possible toxicology properties of turmeric on animals which can induce any side effects upon using it.
- Study the pharmacological properties including active component that represent the anthelmintic properties of turmeric.

REFERENCES

Ahmad W., Hasan A., Ansari A. & Tarannum T. (2010). *Curcuma longa* Linn.- Review. *Hippocratic Journal of Unani Medicinie.* 5 (4): 179-190.

Akram M., Shahab-uddin, Ahmed A., Usmanghani K., Hannan A., Mohiuddin E., *et al.* (2010). Curcuma Longa and Curcumin: A Review Article. *Rom. J. Biol. – Plant Biol.*, 55 (2): 65-70.

Ballweber, L. (2001). *Veterinary Parasitology*. Boston: Butterworth-Heinemann.

Bagchi, A. (2012). Extraction of Curcumin. *IOSR Journal Of Environmental Science, Toxicology and Food Technology*, *1*(3): 01-16. doi:10.9790/2402-0130116.

Hrckova G. & Velebny S. (2013). Parasitic Helminths of Humans and Animals: Health Impact and Control. *Pharmacological Potential of Selected Natural Compounds in the Control of Parasitic Diseases*, SpringerBriefs in Pharmaceutical Science & Drug Development, pp. 29-99.

Kulkarni S.J., Maske K.N., Budre M.P. & Mahajan R.P. (2012). Extraction and Purification of Curcuminoids from Turmeric (*Curcuma longa* L.). *International J. Pharmacology and Pharmaceutical Technology (IJPPT)*, Vol. 1 (2). ISSN: 2277 – 3436.

Martin R.J. (1997). Review: Modes of Action of Anthelmintic Drugs. *The Veterinary.* 154, 11-34.

Martin R.J. & Robertson A.P. (2007) Mode of Action of Levamisole and Pyrantel, Anthelmintic Resistance, E153 and Q57. *Parasitology.* 134: 1093-1104.

Popuri A.K. & Pagala B. (2013) Extraction of Curcumin from turmeric roots. *International J. Innovative Research and Studies.* 2 (5): 290-298.

Schoenian S. (2012). The Internal Parasites that Affect Sheep and Goat. *Merial, Merck Veterinary Manual,* Australian Wool Innovation Ltd.

Singh R., Mehta A., & Shukla K. (2011) Anthelmintic activity of rhizome extracts of *Curcuma longa* and *Zingiber officinale* (Zingiberaceae). *International J. Pharmacy and Pharmaceutical Sciences.* 3(2): 236-237.

Stankovic I. (2004) Curcumin. Chemical and Technical Assessment (CTA) FAO. JECFA.

Sutherland, I., & Scott, I. (2010). *Gastrointestinal nematodes of sheep and cattle*. Chichester, U.K.: Wiley-Blackwell.

Tan T.K., Panchadcharam C., Low V.L., Lee S.C., Ngui R., Sharma R.S.K, *et al.* (2014). Co-Infection of *Haemonchus contortus* and *Trichostrongylus spp*. among livestock in Malaysia as revealed by amplification and sequencing of the Internal Transcribed Spacer II DNA Region. *BMC Veterinary Research.* 10:38.

Ullah S., Khan M.N., Sajid M.S., & Muhammad G. (2013). Comparative anthelmintic efficacy of *Curcuma longa*, *Citrullus colocynthis* and *Peganum harmala*. Global Veterinaria 11 (5): 560-567.

Velayudhan K.C., Dikshi N. & Nizar M. A. (2012). Ethnobotany of turmeric (*Curcuma longa* L.). *Indian J. Traditional Knowledge.* 11 (4): 607-614.

Vidya J, Kale RD & Nair P. (2012). Earthworm muscle Ccells as models to study anthelminitic properties of plant extracts. *International J. Research in Pharmaceutical and Biomedical Sciences.* 3 (2): 489-496.

Watson R.R. & Preedy V.R. (2008). Botanical Medicine in Clinical Practice. *Curcuma longa: a Traditional Herb as an Alternative Medicine.* 671-682. CAB International 2008.

Zeryehun T. (2012). Helminthiosis of sheep and goats in and around Haramaya, Southeastern Ethiopia. *J. Veterinary Medicine and Animal Health* 4(3): 48-55.