P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article

In-Vivo Anthelmintic Activity of the Ethanol Extract of *Allium Cepa* (Onion) Against Mixed Gastro Intestinal Helminth Infestations in Dogs

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Article History: Received: May 20, 2016 Revised: June 16, 2016 Accepted: June 20, 2016

ABSTRACT

Prevalence of animal diseases is one of the major livestock production constraints in Kenya with high impacts on livelihoods due to related economic losses affecting food security in the country. The use of synthetic drugs for disease management has challenges; this makes the use of medicinal plants for treatment a rational alternative. Helminths of zoonotic importance in dogs in Kenya include *Toxocara canis*, *Ancylostoma caninum* and *Dipylidium caninum* which are commonly found in intestines of dogs and can cause infestation in human beings.

This study was designed to evaluate the *in-vivo* efficacy of ethanol extracts from bulbs of *A. cepa* against common gastrointestinal helminths of dogs. Fifteen puppies of mixed sexes, aged between 8 and 10 weeks, with an average weight of 2.2 kg were divided into three groups of 5 animals each; Group 1 was treated with the extract, group 2 was given the recommended dose of a commercial anthelmintic while group 3 was given distilled water, all as single treatments. Fecal samples were obtained from each puppy a day before treatment (day 0) and on days 1, 3, 5, 7, 10 and 14 post treatment for determination of eggs per gram (EPG). Anthelmintic efficacy was determined by calculating the percentage fecal egg count reduction (%FECR) using the pretreatment and post treatment EPG counts. Whole blood was collected from each puppy on days 0, 7 and 14 to determine changes in the hematological parameters. Two puppies from each group were then randomly selected and sacrificed for postmortem examination and for collection of intestinal contents for parasitology.

There was a percentage fecal egg count reduction of 47% for hookworms and a negligible reduction for ascarid worms. There was a significant drop in WBC (P=0.035) 7 days after treatment and a significant increase in RBC (P=0.04) and HGB (P=0.001) 14 days after treatment. The changes in hematological parameters when compared between the treatment and control groups were significant (P<0.05) 7 days after treatment for WBC, RBC, HGB and HCT, and 14 days after treatment for MCHC. There were no signs of toxicity or behavioural changes after oral administration of the *A. cepa* ethanol extract at 6mg/kg. The 47% efficacy against hookworms observed in treated puppies was due to the anthelmintic properties of the crude ethanol extract of *A. cepa*. This is supported by the hematological changes that occurred as a result of administration of the extract.

Key words: Allium cepa, Anthelmintic activity, Gastrointestinal helminths

INTRODUCTION

Most common helminthiases are those caused by infection with intestinal helminths (Peter *et al.,* 2008). The most common intestinal parasites in dogs are ascarid worms and hookworms. The burden of helminth infestation is higher in dogs less than 6 months of age as compared to those more than 1 year (Little, 2009). Classification of helminthes described by Gilbert (1996), is based on the external and internal morphology of egg, larval, and adult stages; adult trematodes (flukes) are leaf-shaped flatworms with prominent oral and ventral suckers that help maintain position in situ. Adult cestodes (tapeworms) are elongated, segmented, hermaphroditic flatworms that inhabit the intestinal lumen, with cystic or solid larval forms that inhabit extra intestinal tissues. Adult and larval nematodes (roundworms) are bisexual, cylindrical worms inhabiting intestinal and extra intestinal

Cite This Article as: A Orengo KO, B Maitho T and Mbaria JM, C Maingi N and D Kitaa JM, 2016. *In-Vivo* anthelmintic activity of the ethanol extract of *Allium Cepa* (Onion) against mixed gastro intestinal helminth infestations in dogs. Inter J Vet Sci, 5(3): 171-175. www.ijvets.com

sites. Helminths of zoonotic importance in dogs include *Toxocara canis*, *Ancylostoma caninum* and *Dipylidium caninum* which are commonly found in intestines of dogs and can cause infestation in human beings.

Treatment and control of intestinal worms is done by providing of anthelmintic drugs. Drugs such as VERMIC TOTAL™ consisting of a combination of Praziquantel, Pyrantel Permoate and Febantel are used in treating ascarid worms and hookworms in dogs. An *in vitro* study of the ethanol extract of *Allium cepa* reported anthelmintic activity against ascarid worms and hookworms of dogs. The extract inhibited egg hatching between 10,000ug/ml and 1,250ug/ml. Chemical compounds found in the crude extracts of *A. cepa* thought to have anthelmintic properties are flavonoids, glycosides, saponins and tannins (Abhijeet *et al.*, 2012).

There are hematological and biochemical changes in animals following worm infestations and analysis of blood parameters is relevant in risk evaluation as changes in the hematological system have higher predictive value for toxicity (Nwaka *et al.,* 2015). A study conducted in dogs infested with *Dirofilaria immitis* found the main hematological changes were anemia in 38.71%, increased numbers of White Blood Cells in 29%, peripheral eosinophilia in 38.71%, basophilia in 35.48%, and decreased hemoglobin (HgB) concentration in 19.35% of the examined dogs (Lefkaditis, 2009). A reverse of some of these parameters is expected by reducing the burden of worm infestation.

The objective of the study was to determine the anthelmintic activity of the crude ethanol extract of *A. cepa* against the common gastrointestinal helminthes; *Toxocara canis, Ancylostoma caninum* and *Dipylidium caninum* in puppies.

MATERIALS AND METHODS

Plant material

Bulbs of *A. cepa* were used for this study. The plant was obtained from Uthiru Market in Kabete, Nairobi and identified at the Kenya National Museums Herbarium in Nairobi.

Preparation of plant extracts

The bulbs were oven dried at 60^{o}C for 48-72 hours, ground to a fine powder, sealed in a paper bag and stored in a cool dry place. The ethanol extract was obtained using methods described by Akintobi *et al.,* (2013). A total of 100g of the dry powder was mixed with 400mls of 99.5% ethanol and agitated several times for a period of 72hrs. The mixture was filtered through a Whatman No. 1 filter paper into a clean beaker and then evaporated over a sand bath at 80^{o}C. Drying was completed in an oven at 40^{o}C in order to prevent the dry extract from sticking to the walls of the container. The dry extract was a maroon colored pasty material with a recovery rate of 2.03%.

Experimental animals

Fifteen mongrel puppies of mixed sexes, aged between 8 and 10 weeks, with an average weight of 2.2 kg were used in the study. The puppies showing signs of natural helminth infection were purchased from households in Ndumbuini village in Kabete, Kikuyu Sub County of Kiambu County. The puppies were vaccinated against parvovirus infection 7 days before admission to the small animal clinic at the Faculty of Veterinary Medicine of the University of Nairobi where they were housed and fed in dog kennels during the experiment. Screening for helminth infection was done by microscopic examination of fecal smears from each puppy. Only puppies with helminth infections were used for the experiment. Natural infection was determined using the fecal smear test.

Determination of in vivo anthelmintic activity

This was done using the fecal egg count reduction test (Coles *et al.,* 2006). A total of 15 puppies were grouped into three groups of 5 animals each. Group 1 was treated with the extract, group 2 was a positive control given the recommended dose of a commercial anthelmintic (Vermic Total™) used for helminthosis treatment in dogs while group 3 was a negative control given distilled water.

The lowest concentration of an ethanol extract which was found to be effective against *A. caninum* and *T. canis* from an *in vitro* study was 1,250ug/ml in distilled water (Orengo *et al.,* 2016). Oral route of administration was selected for administration of the plant extract and distilled water. Each animal was given 10mls. For the positive control group (2), each puppy was given half a tablet of Vermic Total™ orally. Fresh fecal samples were obtained from each puppy a day before treatment (day 0) and for six different days post treatment (days 1, 3, 5, 7, 10 and 14) for determination of the number of helminth eggs per gram (EPG) of feces using the modified Mc Master technique as described by Coles *et al.,* (1992). Anthelmintic efficacy was determined by calculating the percentage fecal egg count reduction (%FECR) using the pretreatment and post treatment EPG counts. The % FECR value was corrected for changes that occurred in the control groups using the formula; % FECR= {1 (T2/T1 X C1/C2)} X 100. Where T and C are the arithmetic means for the treated and control groups and the subscripts 1 and 2 designate the counts before and after treatment respectively (Mbaria *et al.,* 2006).

Determination of hematological changes

1ml of whole blood was collected from each puppy for hematology and analyzed using a haemogram on days 0, 7 and 14 to determine changes in the hematological parameters. Values of White Blood Cells (WBC), Red Blood Cells (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were recorded and statistically analyzed.

Pathological changes

Two puppies from each group were then randomly selected and sacrificed and carefully dissected in order to examine pathological changes as well as to collect intestinal contents in order to quantify and identify adult worms using morphological characteristics.

Statistical data analysis

The mean, range and standard deviation of the fecal egg counts and hematological parameters were

determined. The pretreatment and post treatment EPG values as well as WBC, RBC, HGB, HCT, MCV, MCH and MCHC values for each animal were recorded and entered into the statistical software SPSS from where the statistical analysis was done. The group means for the treatment and control groups were compared by using an independent-samples T test of significance while the pretreatment and post treatment means for each group were compared by using paired-samples T test for significance.

RESULTS

The fecal egg count per gram (EPG) for the puppies before and after treatment for hookworms and ascarid worms are shown in table 1 and table 2 while tables 3 and 4 show pretreatment and post treatment group means and ranges of the EPG values.

Three days after treatment, hookworm EPG counts in the treatment group had dropped significantly ($P<0.05$) while there was no significant drop in the post treatment ascarid worm EPG counts. There was a significant difference ($P<0.05$) in post treatment hookworm EPG counts 7 days after treatment in the negative control group while there was no significant difference in the post treatment ascarid worm EPG counts. There was a significant difference ($P<0.05$) in the treatment and control group means for post treatment hookworm EPG values on the 5th day after treatment, and on the 1st day after treatment for ascarid worm EPG values.

The percentage fecal egg count reduction was 47% for hookworm eggs and a negligible reduction in ascarid worm eggs. No signs of toxicity or behavioural changes were observed in puppies after oral administration of the *A. cepa* ethanol extract at 6mg/kg. Further studies should be done in order to determine if increasing dosage would have more efficacy and also to refine the extract in order to isolate the active chemical compounds and to establish the mode of action of the chemical compounds.

Haematology

Table 5 shows the p values from comparison of the arithmetic means of the monitored haematology parameters between the treatment and negative control

Table 1: Hookworm eggs per gram of feces (n=5) in a study on the efficacy of *A. cepa* ethanol extract against mixed gastrointestinal helminth infestations in puppies

Groups	Animal Id	Days						
		Pretreat	Post Treatment					
		D0	D1	D3	D5	D7	D11	D14
Group 1	G102	750	5700	3100	700	300	960	555
	G103	#	11950	1650	500	5700	1090	#
	G104	3050	#	#	#	2100	2600	1045
	G105	6050	6400	7250	8400	1500	1350	1450
	G106	5650	#	7450	8500	2150	0	#
Group 2	G207	1050	1500	0	0	0	0	0
	G208	3450	0	0	0	0	0	0
	G209	550	#	0	0	#	#	0
	G210	6350	0	#	#	#	1850	0
	G211	#	0	0	0	0	0	0
Group 3	G313	6550	6900	7250	6800	#	7400	7750
	G314	#	8550	9000	8900	9150	9500	#
	G315	2450	2500	2650	2800	#	2700	3350
	G316	1300	2200	2750	3450	3150	3350	3750
	G317	600	1150	1950	2500	2500	#	3100

Key: # means no sample was collected from the puppy

Table 2: Ascarid worm eggs per gram of feces (n=5) in a study on the efficacy of *A. cepa* ethanol extract against mixed gastrointestinal helminth infestations in puppies

Groups	Animal Id	Days						
		Pretreat	Post treatment					
		D0	D1	D3	D5	D7	D11	D14
Group 1	G102	0	50	50	50	0	55	50
	G103	#	550	350	150	200	130	#
	G104	350	#	#	#	0	0	0
	G105	0	400	0	150	50	0	0
	G106	0	#	0	100	50	#	#
Group 2	G207	0	50	0	0	0	0	0
	G208	200	0	0	0	0	0	0
	G209	50	#	0	0	#	#	50
	G210	200	150	#	#	#	0	0
	G211	#	250	0	0	0	0	0
Group 3	G313	150	0	200	0	#	0	0
	G314	#	0	0	310	350	0	#
	G315	165	160	450	0	#	650	0
	G316	0	0	0	0	150	0	0
	G317	0	0	0	0	0	#	150

Key: # means no sample was collected from the puppy

Table 3: Means and ranges of hookworm eggs per gram of feces (n=5) in a study on the efficacy of *A. cepa* ethanol extract against mixed gastrointestinal helminth infestations in puppies

Groups		D0	D1	D3	D5	D7	D11	D14
G1	Mean	8750	8238	4630	4120	2350	1550	1017
	Range	750-6050	5700-11950	1650-7450	700-8500	300-5700	960-2600	555-1450
G2	Mean	2850	1500	0	0	0	0	0
	Range	550-6350	0-1500	0	0	0	0	0
G3	Mean	2725	4260	4720	4890	4933	5738	4488
	Range	600-6550	1150-6900	1950-9000	2500-8900	2500-9150	2700-9500	3100-7750

Table 4: Means and ranges of ascarid worm eggs per gram of feces (n=5) in a study on the efficacy of *A. cepa* ethanol extract against mixed gastrointestinal helminth infestations in puppies

Groups		D0	D1	D3	D5	D7	D11	D14
G1	Mean	87.5	250	100	112.5	60	46.25	12.5
	Range	0-350	50-400	50-350	50-150	50-200	55-130	0-50
G2	Mean	112.5	112.5	0	0	0	0	0
	Range	50-200	50-250	0	0	0	0	0-50
G3	Mean	78.75	32	130	62	166.67	162.5	37.5
	Range	150-165	0-160	200-450	0-310	150-350	0-650	0-150

Table 5: p values from a Levene's test of significance, comparing means of treatment (Group 1) and negative control (Group 3) groups in a study on the efficacy of A. cepa ethanol extract against mixed gastrointestinal helminth infestations in puppies

Parameter	Sampling days (p values)		
	D0	D7	D14
WBC	0.164	0.006	0.437
RBC	0.406	0.050	0.270
HGB	0.815	0.035	0.857
HCT	0.440	0.025	0.255
MCV	0.112	0.534	0.860
MCH	0.802	0.980	0.032
MCHC	0.172	0.809	0.639

Table 6: p values from a Levene's test of significance, comparing means of treatment (Group 1) and positive control (Group 3) groups in a study on the efficacy of A. cepa ethanol extract against mixed gastrointestinal helminth infestations in puppies

Parameter	Sampling days (p values)		
	D0	D7	D14
WBC	0.418	0.004	0.478
RBC	0.338	0.286	0.867
HGB	0.08	0.786	0.509
HCT	0.365	0.249	0.892
MCV	0.008	0.288	0.144
MCH	0.174	0.269	0.280
MCHC	0.768	0.013	0.893

groups. The p values were obtained using the levene's test of significance. Table 6 shows the p values from comparison of the arithmetic means of the monitored hematology parameters between the treatment and positive control groups using the same method.

DISCUSSION

The results obtained from this study indicate that the ethanol extract of *A. cepa* has anthelmintic effect against hookworm infestations in puppies. The reported 47% reduction in EPG counts of hookworm eggs was attributed to the effects of the extract. This demonstrates that administration of the plant extract can reduce hookworm infestation in puppies. However, there was no reduction in the EPG counts of the ascarid worm eggs following oral administration of the extract as expected from the findings of an *in vitro* study of the extract on *Toxocara canis*. *A. cepa* has been shown to have *in vivo* anthelmintic properties. Previously, addition of a combination of *Allium cepa* (onion) and *Cocos nucifera* (coconut) to food of sheep with gastrointestinal nematodes and cestodes was found to stop gastrointestinal infections (Heinz, 2010). The same combination was found to have marked activity against adult *Trichuris muris* and adult *Hymenolepis microstoma* in mice (EP 2 493 490 B1, 2014). No *in vivo* anthelmintic efficacy studies of the crude ethanol extract of *A. cepa* have been documented in dogs.

It was concluded that the 47% efficacy against hookworms observed in treated puppies was due to the anthelmintic properties of the crude ethanol extract of *A. cepa*. This is supported by the hematological changes that occurred as a result of administration of the extract. There was a significant drop in WBC (P=0.035) 7 days after treatment and a significant increase in RBC (P=0.04) and HGB (P=0.001) 14 days after treatment. The changes in hematological parameters when compared between the treatment and control groups were significant (P<0.05) 7 days after treatment for WBC, RBC, HGB and HCT, and 14 days after treatment for MCHC. These hematological changes provide evidence that confirm that administration of the extract reduced worm infestations in the puppies.

Toxicity studies show that the extract is expected to have a safety margin of up to 3,000mg/kg (Salami *et al.*, 2012) body weight. There is a possibility that higher doses maybe more efficacious and hence have more beneficial effects in the management of helminthoses in dogs. Therefore, more efficacy studies using different dose formulations or repeated doses need to be done in order to determine optimal dose and treatment regimens of the plant extract.

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