INTRODUCTION

Gastrointestinal nematode (GIN) infections remain as the main significant impediment affecting the health and welfare of small ruminants worldwide. Nematodes are the cause for both direct and indirect economic losses through decreased productivity, costs of treatment and deaths (Hoste *et al.*, 2008; Kumsa and Nurfeta, 2008; and Várady *et al.*, 2011).

Over the years, parasitic diseases in livestock have been controlled based on the use of broad-spectrum and widely accessible anti-parasitic agents (Coles *et al.*, 2006). In small ruminants, management of parasitic nematodes depends mainly on the application of anthelmintics (Good *et al.*, 2012; Holm *et al.*, 2014; Vadlejch *et al.*, 2014; and Keagan *et al.*, 2017). However, treatment is costly and drug resistance has evolved in all major parasite species of small ruminants (Kaplan *et al.*, 2004; Papadopoulos *et al.*, 2012; Holm *et al.*, 2014; Vadlejch *et al.*, 2014; and Rose *et al.*, 2015), and is severely restrain the potential utilization of this control strategy (Várady *et al.*, 2011; Patten *et al.*, 2011; Keane *et al.*, 2014; Keagan *et al.*, 2017).

In Sudan, the use of anthelmintics in the control of GIN helminths infection in sheep is popular among animal owners. Several pharmaceutical products were registered for the control of helminths in different animal species viz.: Albendazole, Ivermectin, Moxidectin, Levamisole, Tetramisole...etc (NMPB, 2017).

Anthelmintic efficacy can be influenced by many factors; of which, under-dosing, frequent and indiscriminate use of drugs are the important factors that reduce the efficacy of anthelmintics (Patten *et al.*, 2011; Terefe *et al.*, 2013; and Kumar *et al.*, 2013). The use of anthelmintic with substandard quality compounds (Saddiqi *et al.*, 2006; and Kumsa and Nurfeta, 2008) and irrational use of anthelmintics (Patten *et al.*, 2011; and

Terefe *et al.*, 2013) can also influence the anthelmintic efficacy (Menkir *et al.*, 2006). Moreover, smuggling of anthelmintic drugs in many forms, illegal trading in open markets and irrational administration are widespread practice, in Sudan.

For the previous 4 decades Albendazole has been extensively used in Sudan to control gastrointestinal nematodes in large and small ruminant as well as in equines (Imam *et al.*, 2010). In sheep, albendazole showed good efficacy close or near to the threshold of ivermectin (injection) levamisole (bolus), in faecal egg count reduction (100%) (Eldabbagh, 2009). Another study in goat indicated satisfactory efficacy (>95 %) and safety when albendazole was administered at small repeated doses (Hassan *et al.*, 2013). Recently Mohammedsalih and his colleagues (2017) were able to demonstrate *Haemonchus contortus* resistance to albendazole in goats in some areas of South Darfur state by using (FECRT) and egg hatch assay.

Resistance to anthelmintics over years has been evaluated and defined by many scientists according to their findings or susceptibility reduction to anthelmintic medicines, the earliest one was (Prichard *et al.*, 1980) and later on has been adopted by the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) as ‘‘Resistance is present when there is a greater frequency of individuals within a population able to tolerate doses of a compound than in a normal population of the same species, and is heritable’’. The term of treatment failure or resistance is not restrict for nematode solitary but also extend forward to trematode (Brennan *et al.*, 2007).

According to Molento (2009), the use of anti-parasitic medicines in farm animals over the years offers benefits; however, the continuous and inappropriate use of these medicines have resulted in the loss of effectiveness of many active ingredients (Salgado and and Santos 2016).

Although, detection of the unlimited raised wave of resistance throughout the world, but in Africa the situation still under the level of consideration due to negligible numbers of published researches and working in this field (Adediran and Uwalaka, 2015).

The massive and/or indiscriminate use of different anthelmintics namely: albendazole and ivermectin, by animals' owner's may affect wide in the whole map of the treatments in the country and may lead to emergence of anthelmintic resistance, and of course will justify the current work.

Hence, the present study was designed to evaluate therapeutic efficacy of a number of anthelmintics in sheep naturally infected with gastrointestinal nematodes.

In the current study we attempted to investigate therapeutic efficacy of Albendazole 2.5% drench formulation and to compare the obtained results with other commercial drench formulations of Ivermectin .08 %, Tetramisole HCl 5% and Levamisole HCl 2.5% in sheep naturally infected with gastrointestinal helminths in Khartoum State, using faecal egg reduction assay.

So the objectives of the study were to:

i. Assess therapeutic efficacy of Albendazole 2.5% drench formulation against gastrointestinal nematodes using faecal egg count reduction test (FECRT) in naturally infected sheep
ii. Compare obtained results with other commercially available products viz: Ivermectin, Tetramisole, and Levamisole,
iii. Monitor the effect of treatment on liver and kidney function of the treated animals.

CHAPTER ONE
LITERATURE REVIEW

1.1 Helminths infection in small ruminants

Sheep and goats harbour a variety of gastrointestinal tract (GIT) parasites, many of which are shared by both species. Among these parasites, helminths are the most important GIT parasites that affect the growth, production and welfare of the animals.

Helminths infection cause significant morbidity and loss of production in affected animals (Pawel *et al.*, 2004). The infections affect their host health seriously and rival for nutrients in which the predominant clinical symptoms include: loss of weight, stunted growth, compromised immune response, poor feed utilization and conversion, low fertility, condemnation of the affected organs, high prohibitive treatment cost, and in acute non-treated cases may lead to mortality, albeit the infections described as subclinical status; could extend to result in economic losses through high mortality level and decrease in the productivity rate (Waller and Prichard, 1986, Kumsa *et al.*, 2010, and Almeida *et al.*, 2013). However these effects are unapparent to owners because of subclinical or long-lasting chronic infections (Waller *et al.*, 2006).

The effects of gastro-intestinal worms are not restricted to animals but also spread out to human (Regassa *et al.*, 2006, and Adediran and Uwalaka, 2015). The morbidity caused by such infections imposes a substantial burden of disease, contributing to a vicious circle of infection, poverty, decreased productivity, and inadequate or static socioeconomic development.

The animal production especially of the small animals are the first subject to economic attack as a result of the helminths infection, and the

use of anthelmintics is necessary to control these parasitic nematodes (Prichard 1990; Waller, 1997; Gopal *et al.*, 1999), in order to allow welfare and productivity of animals broadly and effectively (Mckellar and Jackson, 2004).

1.2 Anthelmintics

Throughout the universe for many years the application of anthelmintics for the control of small ruminant's helminths is over extensive; this could be due to the ultimate role of helminths in reducing their productivity across the world particularly in developing countries, where nutrition and sanitation are generally referred to as deprived (Balicka-Ramisz *et al.*, 2013).

Anthelmintics regularly have been defined as compounds that destroy or remove helminths from their hosts; could be bi-use for prophylactic measure to prevent the occurrence or as curative for acute or chronic infections. Anthelmintics play a major role in the control of worm burdens; thus enhance animal productivity, decrease shedding of infective worm larvae or eggs to the pasture and reduce environment contamination (Bishop, 2005).

The asymmetric biological characteristics over the different groups of helminths have demanded the discover of alternative anthelmintics groups with atypical mode of actions, roughly to cover these diversities (Bishop, 2005). Since 1960 till 1990s, the manufacturing of anthelmintics has flowered with wide safety margin and broad spectrum (Mckellar and Jackson, 2004). Till 2006 sole four groups of anthelmintics for veterinary use have been detected and rounded in the world (Coles *et al.*, 2006).

Regardless of the dosage forms these anthelmintics common in use in management of parasitic infections in small ruminants, are as follows:

1.2.1 Albendazole

Albendazole is the foremost and widely used anthelmintic in the Benzimidazoles (BZDs) group. Its use is not limited to animals, but extends to include human gastrointestinal parasitic infections.

Albendazole is active against GIT nematodes and has high therapeutic index, the mode of action is mainly through the effect at the microtubules level of the helminths by binding to the growing end thus hinder microtubules from adding new a- ß-tubulin dimers and with the loss of dimers from the other end result in un-stabilized cellular system (Prichard, 2008).

With regards to different species, albendazole has proved to be efficacious in removing helminths from donkeys with 100% efficacy, and no side effects have appeared from administration of the drug to donkeys (Imam *et al.*, 2010).

The evolution of benzimidazole efficacy reduction has been reported worldwide, where in the UK first appeared on sheep (Britt, 1982), followed by many surveys that documented this reduction in efficacy (Bartley *et al.*, 2003). In Germany the first report was in 2001 for *Trichlostrongyle* spp (Bauer, 2001), that is described as a type of multiple resistance. In South America the first report was in 1996 (Waller *et al.*, 1997), in Australia the initial one was in 1986 (Webb and Ottaway), followed by Waller *et al.*, (1995) and Love and Coles, (2002).

In cattle, a report of efficacy reduction was from Poland established by Balicka-Ramisz and Ramisz, (1999). Pashmina goats revealed critical reduction of albendazole efficacy, even when it was used in combination with rafoxanide (Ram *et al.*, 2007). Resistance to albendazole was also reported in horse strongyles, specifically subfamily Cyathostominae which is widely spread phenomenon (Nielsen *et al.*, 2010).

The difference in the absorption-related pharmacokinetic with hold off the feed before the treatment hadn't effect to enhance the efficacy of albendazole against highly resistant nematode of lambs (Alvarez *et al.*, 2010).

1.2.2 Macrocyclic Lactones (MLs)

Macrocyclic lactones are called 3rd generation of broad spectrum anthelmintics developed in the early 1980s and comprises two sub-classes: Avermectins (AVM) such as ivermectin (IVM), abamectin, doramectin, eprinomectin and selamectin; and Milbemycins (MLB) such as Moxidectin (MOX) (Prichard 2008).

Since the discovery of anti-parasitics in 1940s of phenothiazine through oxfendazole in 1970s the administrated dose has decreased at rate of 50%, till innovation of avermectins in 1979 (Burg *et al.*, 1979) which has shifted the administration regime from milligram to microgram per kilogram; almost 25 times lesser.

Diversity of physical and chemical properties of the molecules result in different pharmacokinetics (Mckellar and Benchaoui, 1996). The wide safety margin and good activity of AVM make them favour it for the trait to be used in both humans and animals, extend to reach the agriculture to protect crops, also has prophylactic action and long withdrawal period. Although they have brilliant spectrum against endo- and ecto- parasite but less active against cestodes or trematode (Mckellar & Benchaoui, 1996).

1.2.2.1 Ivermectin

Ivermectin has a structure similar to that of macrolide antibiotics, but without antibacterial activity (Chhaiya, *et al.*, 2012). Ivermectin is a semi-synthetic derivative of avermectin B1 and consists of an 80:20 mixtures of the equipotent homologous 22, 23 dihydro B1a and B1b. This antiparasitic agent, developed by Merck & Co., is frequently used in

veterinary medicine, due to its broad spectrum of activity, high efficacy and wide margin of safety (Fisher and Mrozik, 1989).

Resistance to ivermectin has been reported among ruminants by many researchers in different countries: in UK (Echevarria *et al.,* 1992). In India sub-continent ivermectin was first reported as a resisted medicine by goat GIN by Jaiswal *et al.,* (2013), at the same time efficacy to levamisole was still maintained. Along Indian sub-continent many records have documented reduction of ivermectin efficacy in different animals (Vieira *et al.,* 1992; Miller and Barras, 1994; Ranjan *et al.,* 2002).

Reduction in efficacy of macrocyclic lactones (ML) was also reported in horses and donkeys in the UK (Trawford *et al.,* 2005) and in horses in Kentucky (Lyons *et al.,* 2008). Even though, ivermectin has proved its ability to maintaining the efficacy in goats with higher percentage (Gill, 1996 and Ram *et al.,* 2007).

On the other hand an Indian sheep study proved emerging of resistance to ivermectin (Makvana and Veer, 2009), then was also reported in goats (Jaiswal *et al.,* 2013).

Europe was not so far from the situation, when the first case has been stated out from South-West Britain calves, certainly for *C. oncophora* (Coles and Stafford, 1999). The dramatic reduction in the efficacy of ivermectin is often related to the excessive using in the field due to its wide spectrum against ecto- and endoparasites (Adediran and Uwalaka 2015).

1.2.3 Tetramisole and Levamisole

Particularly Levamisole is the widely used cholinergic anthelmintic. The introduction of levamisole (LEV) use to the sheep industry was at the sixties of the last century, when this had turned the dosing system from gram per kg of body weight to milligram (Mckellar

and Jackson, 2004). There is no difference in the efficacy, bioavailability, milk residues and safety margin of levamisole when it is administrated single or twice effective dose at interval of 10-hours on goats (Chartier *et al.,* 2000).

At the level of nematode its activity at the neuromuscular junction (NMJ), as an agonist at nicotinic acetylcholine receptors (nAChR) resulted in a spastic paralysis. Hence resistance to member of the group present; will spread to other (morantel and pyrantel) and this is usually due to reduction of binding affinity site to the levamisole analogue (Wolstenholme *et al.*, 2004, and Sangster *et al.*, 1998).

Jaiswal *et al.* (2013) reported that levamisole maintained its efficacy against gastrointestinal nematodes in goats; this maintenance could refer to the less regular application of levamisole in this case study.

A considerable number of researches had proved emergence of resistance to Levamisole, where the first report of *H. contortus* resistance in South Africa sheep's was detected by Van wyk and his colleagues, (1989). Many other studies have demonstrated deterioration in the efficacy of levamisole in controlling of GIT parasites in goats (Yadav and Uppal, 1992; Yadav *et al.,* 1995 and Ram *et al.,* 2007). Moreover reduction of efficacy had been documented in India over the last years by (Godara *et al.,* 2011; Manikkavasagan *et al.,* 2015; Jaiswal *et al.,* 2013)

The first report of efficacy reduction for levamisole in Australia (Anderson, 1977) was in cattle *O. ostertagi*, in addition to other two medicines (thiabendazole and fenbendazole). The first documented status of efficacy reduction in *O. ostertagi*, was documented in Belgium cattle (Geerts *et al.*, 1987), a side resistance to morantel tartrate in *O. ostertagi* was demonstrated in Netherlands (Borgsteede 1991), and proved by Sangster *et al.*, (1998), which is also resistant to pyrantel where it share the exact mode of action.

In Denmark the earliest report of levamisole efficacy reduction was in *O. circumcincta* (Maingi *et al.*, 1996b). In cattle levamisole efficacy reduction was also reported in different world regions, Argentina (Caracostantogolo *et al.*, 2005), New Zealand (Waghorn *et al*., 2006) and Brazil (Soutello *et al.*, 2007),

1.3 Anthelmintics Resistance

Resistance has been defined by Shoop *et al.*, (1995) where resistance is determined of whatever medicine by comparison the previously effectiveness status with 95% elimination of the target parasite and become less. Also resistance is considered present if the percentage reduction in faecal egg count (FEC) after treatment was less than 95%, and the lower limit of the 95% confidence interval was less than 90%. If only one of the two criteria was met, resistance was suspected to be present (Coles *et al.*, 1992, and Domke *et al.*, 2012).

Anthelmintics resistance (AR) has become a global problem in the small ruminant industry during the last three decades, with the first cases emerging from the southern hemisphere (Waller, 1994). In Europe, resistance to benzimidazoles (BZs) had been found in up to 80% of flocks and AR to two, or even all three, major groups of anthelmintics has been also recorded (Bartley *et al.*, 2003; Bauer, 2001; Čerňanská *et al.*, 2006; Coles, 1997; Chaudhry, 2015; Maingi *et al.*, 1996a; Sargison *et al.*, 2001; Traversa *et al.*, 2007, Domke *et al.*, 2012).

The importance of resistance to the three groups of broad spectrum anthelmintics has increased dramatically in nematodes of sheep and goats in many parts of the world (Wolstenholme *et al.*, 2004).

Continuous and valuable follow up for the status of efficacy reduction must be performed even in areas of sporadic cases of efficacy reduction (Dolinská *et al.*, 2014), where by tracing reports over the world, the failure of anthelmintics medicines become a global issue which

extend to Europe, Australia and South America and is of increasing importance in certain African countries like South Africa and Kenya (Kumsa *et al.* 2010). However, although anthelmintics resistance to albendazole reached the threshold, but there are some world regions not affected in a manner that can result in ineffectiveness of it. In Europe mainly resistance against benzimidazoles (BZs), in comparison with levamisole resistance and isolated cases of resistance to macrocyclic lactones (MLs) were reported (Papadopoulos, 2008, and Geurden *et al.*, 2014). Notably BZs efficacy reduction has not reached a critical level yet in some European countries, e.g. Greece (Papadopoulos et al., 2001) Spain (Álvarez-Sánchez *et al.*, 2006), Sweden (Höglund *et al.*, 2009), Italy (Rinaldi *et al.* 2014) and Slovakia (Čerňanská *et al.*, 2006).

There are many factors that are related to the animals, to keep anthelmintics efficacy, could be concluded as follows: Updated status of efficacy of currently marketed anthelmintics is basic requirement and first barrier to encounter resistance particularly within regions where medicines are still effective (Dolinská *et al.*, 2014). Unfortunately the absence of new medicines with different mechanism of action makes the AR challenge (Prichard 2008), well management of the recent medicines is needed to keep their efficacy (Coles *et al.* 2006).

Recently the treatment course of anthelmintics has turned to combine chemotherapy with grazing management, but unfortunately resistance interrupt the continuation of anthelmintics use and represent an intimidation for the course. To encounter resistance and prevent it from being spread; knowing of an anthelmintic medicines and means of resistance developed by specific species can advance the effect of these medicines (Gill and Lacey 1998).

The problem of anthelmintic resistance in cattle parasites hasn’t been investigated as intensively as in small ruminants (McKenna, 1996;

De Graef *et al.*, 2013). Assessing the situation in cattle, should be taken into account that in general, cattle parasites are always nearly cause sub-clinical problem. It is possible that even efficacy levels of 50 to 70 percent could well hide the adverse effects of parasites on cattle, so the same frequency of resistant worms in a population might be less likely to be detected in cattle as they are in sheep. However, more and more reports have been published and demonstrated AR in cattle parasites as an emerging problem, with the potential of developing to similar proportions as experienced with small ruminant's parasites (Coles *et al.*, 1998).

1.3.1 Alleles and efficacy association

The unique biological features of some parasites (direct life cycle, short generation and high fecundity) could sympathize the resistant alleles to supersize in the population (De Graef *et al.* 2013). It is assumed that, if resistant parasites have enhanced fitness or if resistance is linked to other fitness genes, the spread of resistance in the population will also increase. Fitness includes all properties that enable more worms to complete their life cycles, such as the egg-laying rate, the persistence of worms in the host, survival on the pasture, the ability to migrate on herbage and their infectivity when ingested (Coles, 2005, Amulya *et al.*, 2015).

There are two hypothesis related to alleles which guide to emergence of decrease potency, the first one is that prior to use the medicines, the alleles already exist within this population (Wolstenholme *et al.*, 2004); the second presumption suggests that the resistance is due to spontaneous and recurrent mutation (Skuce *et al.*, 2010).

The use of medicines is not considered the only motivation for selection of resistance, although the alleles reproductive fitness affect extremely, this could be manifested in the event of absence of

anthelmintics or treatment programs; the resistance alleles will exhibit no or natural reproductively of alleles (Wolstenholme *et al.*, 2004).

Furthermore the type of involved gene affects broadly in efficacy depletion in which the sole and recessive gene prompt efficacy depletion (De Graef *et al.*, 2013, Amulya *et al.*, 2015). Assembly of resistance alleles to a certain drug undergo three phases which are (Wolstenholme *et al.*, 2004):

a) Beginning and continues efficacy loss; usually influenced by many variables such as size and diversity of the population, rate of mutation, adaptation of the animal with the mutation, categorized as low rate.
b) Drug treatment and continued selection of resistant alleles will explode along the affected animals.
c) While continued selection of resistant alleles the status become conscious and reach highest rate.

1.3.2 Types of Resistance

Resistance has many types which have been identified as follow:

1.3.2.1 Side-resistance (within related compounds)

Explicate a type of resistance to certain compound, which has passed from other medicine within the same group and share the exact mode of action, might manifest clearly in BZs, AMs and imidothiazoles (Prichard *et al.*, 1980, Sangster 1999), also could emerge to newly introduced compound despite it hasn't been used before as in Belgian cattle for moxidectin (De Graef *et al.*, 2013). Also widely clear in ivermectin and moxidectin, where many studies have proved that. Regard the potency, moxidectin still in advance of ivermectin to control IVM-resistance species (Kaplan *et al.*, 2007).

1.3.2.2 Cross-resistance (resistance between unrelated compounds)

Resembles side-resistance but involve different groups and different mode of action; that could be clear as in the case of the

organophosphate and the imidazothiazole (LEV) resistance, in which the first one lead to accumulate the acetylcholine at the neuromuscular junction and cause parasite paralysis. This mode of action results in decline of parasite acetylcholine receptors sensitivity to acetylcholine (Sangster, 1999), also multiple or cross resistance to both compounds has also been reported in several cases worldwide (Areskog *et al.,* 2014).

1.3.2.3 Multiple-resistance

Describes the type of efficacy reduction; regularly involving 2 or more different anthelmintics groups, arising from use of each group alone or from cross resistance (Prichard *et al.*, 1980), recently the prevalence of Multiple Drug Resistance (MDR) increased to an incredible limits worldwide to involve not only the small ruminant but extend to cattle and horses, but doesn't reach the same levels (Kaplan, 2004 and Gelot *et al.*, 2016).

Multi - drug efficacy reduction has been reported worldwide, as in Brazil, where it is reported in sheep farms for albendazole (ABZ), levamisole (Leva), combination of Leva with ABZ and ivermectin (90%, 84%, 73% and 13%) respectively (Echevarria *et al.*, 1996). Also, in ten herds of Nigerian cattle, by using Larval Development Assay (LDA) for four medicines (albendazole, febantel, levalmisole and morantel) (Fashanu and Fagbemi, 2003). Also in goat multidrug efficacy reduction has been documented in 15 Danish goat herds by using faecal egg count reduction test (FECRT), egg hatch assay (EHA) and larval development assay (LDA), where 6 farms have revealed reduction for BZs and LEV, and for IVM and BZs on one farm. On the other hand, reduction appeared for BZs and LEV in two different farms, and one farm for IVM (Maingi *et al.*, 1996b). In addition to France, multiple resistances to levamisole (LEV) have been detected in goats (Paraud *et al.*, 2009).

Strategies to Combine medicines from the same or different anthelmintics groups to fortify and widen the spectrum; have also shown well advance in efficacy reduction, as in sheep in New Zealand, where benzimidazole (BZ) displayed in 60% (9/15) of the farms, to levamisole (LEV) in 66% of farms (10/15), combination drench (BZ+LEV) on 43% of farms (3/7) and avermectin on 1 of 8 farms (Sharma, 2004).

Also combination (ABZ + TET), in addition to ABZ, TET and IVM in nematode of sheep and goats, has demonstrated well efficacy in eastern Ethiopia (Sissay *et al.*, 2006), but in another study some years later in Southern Ethiopia with the same medicines has defeated this (Sheferaw *et al.*, 2013).

When restricting the area to Rogaland County, eight flocks out of ten (80%) non-randomly selected sheep flocks showed BZ resistance. The efficacy of ML was 100% in all surveyed sheep and goat flocks. In post-treatment coprocultures from the non-randomly selected flocks, the main nematode genera were *Teladorsagia*/*Trichostrongylus* in five flocks, *Haemonchus* in two flocks, and a mixture of these genera in the remaining two flocks. In the goat flocks, the pre- treatment infection levels of GIN were low compared to what was found in the sheep flocks. Still, in one flock, AR against BZ in *Teladorsagia*/*Trichostrongylus* was found (Domke *et al.*, 2012).

Kaminsky *et al.*, (2008) concludes that farms in the western region of Santa Catarina have anthelmintic resistance to closantel, albendazole, and Levamisole; i.e. the FECRT was less than 95% in all farms investigated. The anthelmintic resistance of the genera *Haemonchus* and *Trichostrongylus* was observed in the farms. In contrast, no resistance was observed to *Teladorsagia* spp., *Cooperia* spp. and *Oesophagostomum* spp. at this time.

Melaku *et al.,* (2013) has conducted study in North Western Ethiopia, targeted ABZ, IVM, TET, LEV, IVM+ABZ and ABZ+LEV in 28 naturally infected sheep, where reported excellent efficacy of combined medicines groups (IVM+ABZ and ABZ+LEV) (100%) and the least one was in TET group (89.51 %), and between scattered others groups; ABZ (99.08 %), IVM (96.69 %) and LEV (90.06 %).

1.3.3 Phases of worm resistance

Once the trigger of resistance starts in a herd, there is no way to reverse the situation, although loss of efficacy present in many phases in which could be controlled in each. These phases initially don't visualize at the first use of anthelmintics which is considered effective against the phenotype susceptible parasites, with decrease in the number of affected parasites later without alteration in treatment course. The heterozygous individual resistance expands within the herd, eventually extend to evolve the homozygous and resistance explode dramatically. Roughly the rate of progress depends upon the regularity of treatment program and over usage per year regardless of the epidemiology situation. Furthermore could involve other groups (FAO, 2004, Amulya *et al.*, 2015).

There are many factors resulting in the anthelmintics efficacy loss, which could conclude as follow: misusing factors, either for the same group of anthelmintics or inherited from others, might be sporadic or combined cases and manifested as:

a) Sub- optimal doses; affect drug bioavailability which is present at a lower rate than animal needs, (De Graef *et al.*, 2013), and is obvious at goat with BZ and macrocyclic lactone (MLs) where they are treated at the dose of sheep or cattle despite actually required dose (Bogan *et al.*, 1987; Chartier *et al.*, 1999; Hennessy *et al.*, 1993; Hennessy and Alvinerie, 2002; Sangster *et al.* 1993; Short *et al.*, 1987; and Domke *et al.*, 2012),

b) Gradual excess of dosing will result in decline of efficacy (Dolinská *et al.*, 2014),
c) Prophylactic treatment of domestic animals (higher and repeated doses) ((Wolstenholme *et al.*, 2004),
d) The geographic location and the climatic weather have noticeable effect in the loss of anthelmintics efficacy and uprising of resistance in sheep and goat due to the factors have mentioned above (Kumsa and Wossene, 2006),
e) Management factors such as in gating and grazing in the same place as well as share the same parasites species usually progress to participate in the status of AR as manifested in sheep and goat (Coles *et al.*, 1996),
f) Lack of new formula (Hamdullah *et al.*, 2014).

Drug resistance of parasitic helminths is becoming a serious problem in veterinary medicine, especially in sheep husbandry (Roos *et al.*, 1993). In comparison with cattle the situation is not far away which has presented in many investigations and the factors is likewise similar to small ruminants (Sutherland and Leathwick, 2011), or could associate with exclusive other factors (De Graef *et al.*, 2013). All these factors have contributed to the widespread development of anthelmintic resistance (AR) (Shalaby, 2013, Amulya *et al.*, 2015).

1.3.4 Detection and diagnostics methods for anthelmintic resistance

With the development and spread of AR in nematodes of livestock, the need for methods to detect resistance has evolved simultaneously. A wide range of tests have been developed to detect AR for research and diagnostic purposes (Presidente, 1985; Amulya *et al.* 2015). There are two ways for diagnosis of AR as follows:

1.3.4.1 *In vivo* diagnostic methods of anthelmintics resistance

Faecal egg count reduction test for sheep and goats (FECRT): is the first and the most commonly practiced test to study anthelmintic resistance (Presidente, 1985). This test was originally designed for sheep, but can be used also for cattle, swine and horses. This test is particularly suitable for field surveys and it has the advantage that the number of groups can be increased if appropriate, to test the efficacy of a range of broad or narrow spectrum anthelmintics at one time. For monitoring of normal fluctuation, the treated group is generally compared with non-treated controls (FAO, 2004).

This test is easy to perform, suitable for ruminants, horses and pigs as well as for all types of anthelmintic. In addition, it can be carried out on any species of nematodes in which eggs are shed in the faeces. This test estimates the efficacy and resistance by comparing egg counts before and after the treatment (Gill *et al.*, 1998).

The sub limitation one for the use of FECRT is the loss of sensitivity of modified McMaster technique to detect lower eggs, and cannot detect egg <50, for that reason other technique is suggested such as FLOTAC technique, with a detection limit of 1-2 EPG (more sensitivity and accuracy) (Rinaldi *et al.*, 2011). Also non species-specific and difficulty to microscopically differentiated between nematodes eggs; to bypassing this larvae culturing is suggested (De Graef *et al.*, 2013).

The FECRT is still considered as the most practical and direct way of detecting nematodes resistant to BZ and ML in sheep and goats (Cabaret, 2004), although it has a low sensitivity, being able to detect AR only in populations where more than 25% of the worms are resistant (Martin *et al.*, 1989, Domke *et al.*, 2012), another possible critical issue is the occurrence of low excretion of nematode eggs and the large confidence intervals sometimes associated with the FECR calculations.

Techniques with a high-analytic sensitivity are preferred for monitoring drug efficacy in populations with low fecal egg excretion (Levecke *et al.*, 2011).

FECRT Method (Coles *et al.*, 2006): is done according to the following procedure:

1. Randomly distribute or distribute based on egg counts.
2. Choose animals 3–6 months of age or if older with eggs counts >150 epg.
3. Use 10 animals per group (if possible).
4. Rectal sample putting 3–5 g into individual pots.
5. Count using the McMaster technique as soon as possible after collection.

Calculation of the total number of eggs per gram of faeces is calculated using the following equation:-

$$\text{Number of eggs/gram of faeces} = \frac{\text{Number of eggs counted x total volume of mix (ml)}}{\text{Volume of counting chamber (ml) x wt of faeces in mix}}$$

6. Only store at 4°C for 24 h if using samples for culturing.
7. Individually weigh animals and give manufacturers recommend dose orally, from a syringe.
8. Take second rectal sample at the following time periods after treatment: Levamisole 3–7 days, Benzimidazole 8–10 days; and Macrocyclic lactones 14–17 days.
9. If testing all groups in same flock, collect on day 14.

1.3.4.2 *In vitro* diagnostic method of anthelmintics resistance

Molecular techniques for anthelmintics resistance

A number of different approaches to the measurement of genetic variation are available involving either the direct analysis of gene polymorphism by using restriction fragment length polymorphism or

analysis of electrophoretic variation in gene products by a wide range of electrophoretic techniques. The analysis of the electrophoretic mobility of a range of enzymes by starch gel electrophoresis can provide a genetic profile which can be used to identify strains within a species (Nomura, 1984). Such analysis has given useful information on the characterization of strains and species of a large number of parasites, for example, the identification of zoonotic trypanosome subspecies (Tait *et al.*, 1985) and the identification of benzimidazole -resistant strains of nematodes (Sutherland *et al.*, 1988). The latter authors showed differences in the esterase patterns between benzimidazole-susceptible and benzimidazole-resistant strains of *Haemonchus contortus* (Echevarria *et al.*, 1992).

There are other methods considered extra accurate than *in vivo* test (FECRT) because of no animal-interference, and reasonable cost, furthermore have the ability to be repeated and standardized (Sangster and Gill, 1999).

a) *Larval migration inhibition assay (LMIA)*: based on the drug induced paralysis of the body musculature of Trichostrongyloid nematodes;
b) *Micro- motility meter test (MMT)*: the main concept depends on incubation in anthelmintic dilutions, fractionates light rays, and measured with a photodetector. The numerical representation of this signal is termed the motility index. Active worms give higher indices than paralyzed worms (Folz *et al.*, 1987; Demeler *et al.*, 2010).
c) *Larval development assay (LDA)*: measures the potency of the anthelmintic as inhibitor of the development of starvation through the inhibition of feeding;
d) Larval feeding assay (LFA); and
e) Egg hatch assay (EHA):

The Egg hatch test or assay: is used to validate the result of FECRT which is recommended by W.A.A.V.P. guidelines to estimate the ability

of the eggs to hatch at series of thiabendazole (Coles *et al.*, 2006). It is based on the determination of the proportion of eggs that fail to hatch in solutions of increasing drug concentration in relation to the control wells, enabling the user of the test to develop a dose response line plotted against the drug concentration. To obtain meaningful data, eggs for the egg hatch test must be fresh and should be used within three hours of being shed from the host, as sensitivity to some benzimidazoles decreases as embryonation proceeds. The test has only been shown to work on nematode species in which eggs hatch rapidly (FAO, 2004).

The advantage of this test is that a single faecal sample can be tested simultaneously for all available classes of anthelmintics (Fleming *et al.*, 2006).

The use of discrimination/delineating dose (DD) in the EHT provides a good estimate of genotypic resistance. The egg hatch discrimination dose test (EHDDT) is less time-consuming, allows the reliable detection of a frequency of resistance alleles below 10% and is fairly reliable for the detection of BZ resistance under field conditions (Čudeková *et al.*, 2010).

Larval development test: of all the available tests, the larval development test is the most sensitive for quantitatively measuring thiabendazole and levamisole resistance (Al-hasnawy, 2014).

1.4 Effect of Route of drug administration on drug efficacy

For most anthelmintics, efficacy is related directly to duration of the contact between drug and parasite. To ensure sufficient drug-parasite contact time, it is important that the full dose lodges in the rumen where the drug then binds to rumen particulate matter and is slowly released as digesta passes down the digestive tract. Sangster *et al.,* (1991), reported that, presenting a drench to the buccal cavity, rather than into the pharynx and esophagus, can stimulate closure of the esophageal groove with a

large amount of the drench bypassing the rumen. Therefore, oral anthelmintics should always be administered using a properly designed drenching gun, or using a syringe with a drench adapter. Once in the rumen, duration of drug availability as it flows to more distal sites of absorption is largely dependent on flow rate of the digesta (Hennessy, 1997).

Differences have been reported in the plasma concentrations of anthelmintics, depending on the dietary intake of dry feed or grazing (Ali and Chick, 1992). Withholding of food from animals overnight before drenching may increase the efficacy of benzimidazole anthelmintics, but not ivermectin or levamisole (Ali and Hennessy, 1995, Escudero *et al.*, 1997, Charlier *et al*., 1997).

Chapter Two

Materials and Methods

2.1 Study location

This study was conducted in the Farm of the College of Veterinary Medicine, Sudan University of Science and Technology. The farm is located in Hillat Kuku, East Nile Locality, Khartoum State, Sudan.

2.2 Experimental animals

A total of 16 male sheep (local breed) was used in the current study. They were 8-12 months of age. Animals were purchased from *Elaaelafoon* market, East Nile Locality, Khartoum State, Sudan. Animals were kept for 2 weeks before the start of the experiment in order to be adapted with the surrounding environment in the farm.

2.3 Animal housing and feeding

Animals were housed individually, in pens with dimensions of 2X1.5 meters, during the experimental part of the study. Animals were provided with sorghum and calculated amount of Dura maize, and allowed with tap water *ad libitum*.

2.4 Experimental drugs

Four different drugs were used in the current study viz.:

1. Albendazole 2.5% drench formulation: Albendazole® 25 mg suspension from Kela Laboratoria NV, Belgium.
2. Ivermectin 0.08 % drench formulation: Intermectin® 0.8 mg oral solution from Interchemie werken ‘‘De Adelaar’’, Holland.
3. Tetramisole 5% drench formulation: Tetrapam-L® 5% oral solution from Bash Pharma Pharmaceutical, Sudan.
4. Levamisole 2.5% drench formulation: Levozide® Drench 2.5% suspension from

   Punjab Drugs House (P. D. H) Laboratories, Pakistan.

2.4.1 Drugs testing

All drugs utilized in the current study were subjected to chemical analysis before the start of the experimental part of the study to detect finished product quality (assay) and assure complying with specification. Methods used were either Pharmacopeial method (BP) or manufacturer methods (in house). All drugs were analyzed using High Performance Liquid Chromatography (HPLC) (Shimadzu vaphy Shimadzu, liquid chromatography with UV/visible and decide ray-detector, isocratic and low pressure gradient pump and pc control; Japan). Drugs analysis was conducted in the National Medicines Quality Control Laboratory, Khartoum, Sudan.

2.4.2 Drug samples selection criteria and collection

Drug Samples were selected according to National Medicines and Poisons Board (NMPB) importation rate along the year of the study, and collected from different private Veterinary pharmacies in Khartoum state. Drugs were stored at temperature about 30ºC according to (NMPB) guidelines for (GSP).

2.4.3 Drugs analysis methods

2.4.3.1 HPLC Method for analysis of Albendazole (BP, 2016)

An amount equal to 0.0208gm of Albendazole working standard was weighed and dissolved in 100 mL mixture of Sulphoric acid: Methanol (1:100) (w/v). 5mL of the sample (equivalent to 125mg) was transferred to 100mL volumetric flask where dissolved in a mixture of Sulfuric acid: Methanol (1:100) (w/v), stirred for 15 minutes and sonicated for 10minutes to be mixed, then allowed to stand and the supernatant was taken to make another dilution at (1:5) with the same above solvent. As a mobile phase Ammonium dihydrogen orthophosphate (Mobile phase A) 3.4gm and Methanol HPLC-Plus gradient (Mobile phase B) 600mL were used, with flow rate of

0.7mL/minute. The liquid chromatography was equipped with 292 nm and a column C18: (25cm* 4.6mm). The injection volume was 20µL of standard and sample solutions (BP, 2016).

2.4.3.2 HPLC Method for analysis of Ivermectin (non-Pharmacopeia)

An amount equal to 0.0202gm of ivermectin working standard was weighed and dissolved in 5mL of methanol, 2mL of the solution was taken and completed to 10mL with the same solvent to obtain concentration equivalent to 8µg/mL. A volume of the sample equivalent to 8mg was transferred to 10mL volumetric flask where dissolved with 10mL of Methanol, stirred for 15 minutes and mixed with the aid of ultrasound for 10 minutes, a solution with concentration equivalent to 8µg/mL was obtained. As a mobile phase a mixture of (Water: Methanol: Acetonitrile) 120:240:640 (v/v/v), with a flow rate of 1.0mL/minute was used. The liquid chromatography was equipped with 254 nm and a column C18 (150cm*4.6mm) and oven with 30 ºC. The injection volume was 20µL for standard and sample solutions (Interchemie werken ‘‘De Adelaar’’, Holland).

2.4.3.3 HPLC Method for analysis of Tetramisole content (non-Pharmacopeia)

An amount equal to 0.0312gm Tetramisole HCl working standard was weighed and dissolved in 25 mL of methanol, another dilution was made with solvent of (water: methanol) (50:50) at (2:100); to obtain a solution with a concentration equal to (25µg/mL) of Tetramisole base. 5mL of the sample (equivalent to 250 mg Tetramisole HCl) was transferred to 50 mL volumetric flask and diluted with 50 mL methanol and shake. 5mL of the solution was transferred to 50 mL volumetric flask and diluted with the same solvent (50 mL). Another dilution was made with other solvent (water: methanol) (50:50) at (1:100), to obtain a solution with concentration (25µg/mL) of Tetramisole base. As a mobile

phase a mixture of Methanol, Water, Heptane, sulfonic acid and Phosphoric acid (300:250:0.75:2.5) (v/v/w/v) was used with a flow rate of 1.0mL/minute. The liquid chromatography was equipped with 225nm and a column C18: (250cm*4.6mm) and 5µ particle size. The injection volume was 10µL of standard and sample solutions (Bash Pharma Pharmaceutical, 2017).

2.4.3.4 HPLC Method for analysis of Levamisole HCl (non-Pharmacopeia)

A quantity of Levamisole HCl working standard was weighed and transferred to a volumetric flask where dissolved with distilled water to obtain a solution with concentration of 30µg/mL. A volume of the sample equivalent to 8mg of Levamisole HCl was transferred to volumetric flask where dissolved with distilled water to obtain a solution with concentration 30µg/mL. As a mobile phase a mixture of phosphate buffer Ph 8: Acetonitrile (70:30) % with flow rate of 1.0 mL/minute was used. The liquid chromatography was equipped with 215 nm and a column C8 was used. The injection volume was 10 µL. The flow rate was 1.0 mL/minute for standard and sample solutions (Amal, 2018).

2.5 Animals grouping and treatment protocol

Two experiments were conducted in the current study.

2.5.1 The first experiment:

Sixteen male sheep, 8-12 months of age were used in the faecal egg count reduction test. Animals were selected based on apparent clinical signs of GINs infection, such as; weakness, loss of hair, lack of appetite, marked loss of body weight, history of reduced production, sub-mandibular oedema and anaemia, and finally confirmed with laboratory examination of positive GINs infection using modified McMaster technique where only sheep with (> 150) egg per gram were utilized for this study. On day zero, faecal samples were collected and the sheep were

assigned into two treatment groups each of eight animals. Animals were weighed in order to estimate the dose. Animals in the first group (A) were drenched according to body weight with albendazole (Albendazole 2.5%) orally by syringe at 5mg/kg body weight as single dose. While animals in the second group (B) were treated with ivermectin (ivermectin 0.8%) 0.2mg/kg body weight. To avoid under dosing, dose volumes were rounded up to the nearest 0.5ml. Further faecal samples were collected at days 2, 3, 4, 7, 10, 14, and 21 for animals in the two groups (A and B) post-treatments.

2.5.2 The second experiment:

After completion of the first experiment, and due to the failure of the treatment to eliminate GINs infection, animals were then allowed for 7 days before rearrangement of the previous groups into two treatment groups (C) and (D) each of eight animals, according to previous procedure adopted in the first experiment (equal distribution of egg count within the two groups). Animals in group (C) were drenched orally with Tetramisole (Tetramisole 5%) at dose rate of 3mg/kg bwt, while animals in group (D) were treated orally with Levamisole (Levamisole 2.5%) at 7.5mg/kg bwt according to manufacturer recommended dose. Animals were sampled for epg (egg per gram) count before treatment at day 0, and then following treatment at days 2, 3, 4, 7, 10, 14, and 21.

2.6 Parasitological methods

2.6.1 Faecal samples collection:

Faecal samples were collected directly from the rectum in clean plastic containers and labelled and transported immediately to the diagnostic laboratory, Department of Animal Health and Surgery, College of Veterinary Medicine (SUST). Samples were collected in the morning from 8:00 to 9:00 AM.

2.6.2 Modified McMaster technique (Egg count): procedure

Egg count was performed using modified McMaster technique where each egg counted represents 50 eggs per gram of faeces (Stafford *et al.*, 1994) as following:

1. Three grams of faeces were mixed with 42 ml of tap water and the faecal suspension was passed through the 80 μm square sieve to remove debris.
2. The filtrate was collected in a clean dry container.
3. 15 ml of this filtrate was taken into a centrifuge tube and centrifuged at 1500 rpm for 2 minutes and the supernatant was then discarded.
4. The sediment was emulsified by gentle agitation and saturated NaCl was added until the volume became equal to the initial aliquot of the filtrate.
5. The centrifuge tube was inverted several times to obtain an even suspension of the contents.
6. The two chambers of the McMaster slide were filled using a clean Pasteur pipette.
7. The average number of eggs present in the chambers was multiplied by 100 to obtain the number of egg per gram of faeces (epg).

2.6.3 Coproculture (larvae identification):

Larvae were identified according to Baerman technique (MAFF, 1977), faecal samples were collected 7 days before the start of the experimental part of the study using sterile disposable plastic gloves directly from the rectum in labelled clean plastic containers, and then dispatched to the laboratory. Pooled faecal samples were mixed with water and kept at 37°C for approximately 14 days to allow development to the third larval stage, then transferred to funnel with distilled water where protected with gauze to prevent from being diluted, and left over

night to enable migration of the larvae throughout the gauze, the sediment centrifuged for 3000rpm/3minutes, and the precipitated was stained with iodine and examined microscopically. 100 larvae were identified based on morphology. Larvae were identified using the keys provided by Anon (1986).

2.6.4 Calculation of therapeutic efficacy:

Eggs per gram of faeces (EPG) of the animals in all groups were counted at day 0 (Pre-treatment) and days 1, 3, 7, 10, 14 and 21 (Post-treatment) using McMaster technique. The efficacy of the drugs was calculated as per formula described by Faecal egg count reduction (FECR) was calculated using the method endorsed by the World Association for the Advancement of Veterinary Parasitology (Coles *et al.*, 1992).

$$\text{Efficacy (FECR) \%} = \frac{\text{(Pre-treatment epg-Post-treatment epg)}}{\text{Pre-treatment epg}} \times 100$$

Resistance is considered to occur when the FECR was <95%.

2.7 Collection of blood samples

Blood samples for serum were withdrawn from the jugular vein in syringes, following transfer to plastic containers; they were allowed to clot at room temperature. The clotted blood samples were centrifuged and sera were separated and stored at -20 ºC until analyzed.

2.8 Methods of determination of blood biochemical parameters in sheep treated with different anthelmintics

2.8.1 Total protein determination method

Total protein was analysed by Biuret method (King and Wooton, 1956) using a commercial kit (Vitro scient, Egypt). Cupric ions, in alkaline medium, interact with protein peptide bonds resulting in formulation of a coloured complex. The optical density of the developing

colour was measured at 546 nm using spectrophotometer (Jenway 6305 UV/Vis, UK).

2.8.2 Albumin determination method

Albumin was detected by colorimetric endpoint method following the modified bromocresol green binding according to Bartholomew and Delany, (1966). In this method Albumin at pH 4.2 bind to bromocresol green and form blue green coloured complex, the intensity of this colour is proportional to the albumin concentration and determined by measuring the increase in the absorbance at 580 nm using spectrophotometer (Jenway 6305 UV/Vis, UK) and commercial kits (Vitro scient, Egypt).

2.8.3 Alanine aminotransferase (ALT) determination method

ALT activity was detected by Kinetic UV method which was first described by Henley and Pollard (1955) and modified by Reitman and Frankel, (1957). In this method the amino group is enzymatically transferred by ALT present in the specimen from alanine to the carbon atom of 2 oxoglutrate yielding pyruvate and L-glutamate. Pyruvate is reduced to lactate by LDH present in the reagent with the simultaneous oxidation of NADH to NAD. The rate of oxidation of NADH is proportional to ALT activity in the specimen, and it was determined by measuring the decrease in absorbance at 340 nm using spectrophotometer (Jenway 6305 UV/Vis, UK) and commercial kits (Vitro scient, Egypt).

ALT activity was calculated by determining the change in absorbance per minute (ΔA/min) from the linear portion of the reaction curve and the ALT activity was calculated using the following formulae

U/I= 1746× ΔA/min.

2.8.4 Aspartate aminotransferase (AST) determination method

Aspartate aminotransferase (AST or GOT) catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate, forming oxalacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH; measured at 340 nm, by means of the malate Dehydrogenase (MDH) coupled reaction according to Reitman and Frankel, (1957).

The working reagent and the instrument were brought into reaction temperature. The serum was collected by standard procedures. Then the required amounts of serum, standard solution and distilled water (blank) were prepared in the cuvettes.

The cuvette content was mixed and inserted into the spectrophotometer (Jenway 6305- U.V/VIS. spectrophotometer UK). The stopwatch was started.

After 1 minute interval, the initial absorbance was recorded and at 1 minute intervals thereafter for 3 minutes. The difference between consecutive absorbance was calculated, and the average absorbance difference per minute (ΔA /min).

The AST/GOT concentration in the sample was calculated using the following general formula:

U/I= 1746× ΔA/min.

2.8.5 Urea determination method

Urea in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry (Fawcett and Scott, 1960).

The serum was collected by standard procedures. Then tubes containing the required amounts of serum, standard solution and distilled water (blank) were prepared. Spectrophotometer (Jenway 6305- U.V/VIS.

spectrophotometer UK) was used; the absorbance (A) of the standard and the sample were Red colour at 600 nm against the Blank.

The urea concentration in the sample was calculated using the following general formula:

$$\frac{A\ Sample}{A\ Standard} \times C\ Standard \times Sample\ dilution\ factor = C\ Sample.$$

2.8.6 Calcium determination method

Calcium in the extracellular fluid is critical for normal neuromuscular excitability, capillary and membrane permeability, normal muscle contraction, normal transmission of nerve impulses, and normal blood coagulation (Kaneko *et al*., 1997).

The serum calcium concentration was determined by photometric colorimetric test-cresolphthalein method kit (Biosystems S.A., Spain). Calcium ions form a violet complex with o-cresolphthalein complexone in alkaline medium. The intensity of the developing colour was measured at 570 nm using Jenway spectrophotometer (Jenway 6105 U. V. /vis. Spectrophotometer, U. K.). The calcium values were calculated in mmol/l of serum according to Sarkar and Chauhan (1967) and Barnett *et al*., (1973).

2.8.7 Phosphorus determination method

Inorganic phosphorus reacts with ammonium molybdate in acid medium to form a phospholybdate complex which absorbs light at 600-675 nm. The absorbance at this wavelength is directly proportional to a mount of inorganic phosphorus present in the sample.

The serum was collected by standard method. Then the tubes containing the required amounts of serum or standard solution were prepared.

The tubes contents were mixed and incubated for 15 minutes at room temperature (+15-25ºC). Spectrophotometer (Jenway 6305-

U.V/VIS. spectrophotometer UK) was used, the absorbance (A) of the Standard and the Sample were measured at 600-675 nm against the Blank.

The final concentration of phosphorus in the sample was calculated using the following general formula:

$$\text{Serum inorganic phosphorus mg/dl} = \frac{\text{A Sample}}{\text{A Standard}} \times 5$$

2.9 Statistical analysis

Values obtained were tested for significance using t-test to compare pre-treatment (baseline) with post treatment values for the different parameters tested using Graph pad prism package for statistical analysis.

CHAPTER THREE

RESULTS

3.1 The first experiment

3.1.1 Drug testing:

The two drugs utilized in the first experiment (Albendazole and Ivermectin) showed assay% values that comply with the specifications for registration at the Directorate of Veterinary Medicines Registration, in the National Medicines and Poisons Board (NMPB), Sudan (Table 3.1).

3.1.2 Larval identification:

Strongyloides papillosus and *Haemonchus* spp. comprised 84% of the larvae identified in the infected sheep (Table 3.2)

3.1.3 Faecal egg count reduction:

The results of day zero to day 21 epg values are presented in Table (3.3), together with the mean faecal egg count reductions. At day 14, Albendazole and Ivermectin produced 33.8% and 48.5% reduction in faecal egg count, respectively. It is worth to mention that up to the end of the experiment at day 21 all animals had epg equal to or greater than 300/epg.

Table 3.1: Assay (%) of Albendazole and Ivermectin

Generic name/ Dosage form	Type		Average retention time	Area	Sample			
					Reference	Specification	Sample Content % (N=3)	RSD %
Albendazole oral suspension	Standard		21.867	14277591	BP 2016	90-110%	108.9	0.543
	Sample		21.937	16530224				
Ivermectin oral solution	Standard	H_2B1b	12.43	526283.333	Interchemie werken	90-110%	100	0.1071
		H_2B1a	15.376	17725310				
	Sample	H_2B1b	12.5187	525623				0.0242
		H_2B1a	15.498	17731147				

Table 3.2: larval identification of nematodes obtained from pooled faecal samples of sheep

Type	Number	Percentage
Haemonchus contortus	1300	26
Oesophagostomum spp	100	2
Strongyloides papillosus	2900	58
Hookworms,	50	1
Dictyocaulus spp.	150	3
Protostrongylids	100	2
Nematodirus spp	400	8

Table 3.3: Mean faecal egg count (arithmetic) and reduction (%) for Albendazole and Ivermectin-treated sheep

Days	Albendazole		Ivermectin	
	epg (Arithmetic Mean)	Reduction %	epg (Arithmetic Mean)	Reduction %
0	981.3	-	1625	-
2	250	74.5	725	55.4
3	475	51.6	975	40.0
4	262.5	73.2	975	40.0
7	100	89.8	1725	-6.2.0
10	412.5	58.0	1413	13.1
14	650	33.8	837.5	48.5
21	837.5	14.6	1400	13.8

3.1.4 Safety of Albendazole and Ivermectin administration in sheep naturally infected with GINs

There was no significant change in total proteins concentration at day 7 in both treatment groups following administration of treatments. While there was sharp significant ($P<0.05$) decrease in albumin concentration in animals treated with albendazole at day 21 following treatment (Table 3.4).

There was no significant increase in the level of ALT enzyme at day 7 post treatment in the two animal groups. The level was almost the same to that of the pre-treatment values at days 14 and 21 post treatment. There was significant ($p<0.05$) decrease in AST level in the two treatment groups at day 7 post treatment. The level of the enzyme returned to almost the same level at pre-treatment day with no significant difference with day zero (Table 3.5).

There was no significant change in urea concentration in the two treatment groups following administration of albendazole and ivermectin (Table 3.6). Calcium concentration increased significantly ($P<0.05$) following treatment with albendazole at days 14 and 21 post treatment with albendazole. In the same line there was significant increase ($P<0.05$) in calcium concentration following treatment with ivermectin at days 7 and 21 following treatment.

There was significant ($P<0.05$) decrease in phosphorus concentration at days 7, and 21 in the animals treated with albendazole. While in the animals treated with ivermectin there was significant ($P<0.05$) increase in phosphorus concentration at day 7 post treatment (Table 3.7).

Table 3.4 : Effect of treatment of sheep naturally infected with GINs with albendazole and Ivermectin on total proteins and albumin concentration (N=8)

Parameters	Total proteins (g/L)				Albumin (g/dl)			
Treatment	Albendazole		Ivermectin		Albendazole		Ivermectin	
Days	Mean±SD	P value	Mean±SD	P value	Mean±SD	P value	Mean±SD	P value
0	68.62±9.20		61.67±7.60		48.07±12.60		25.33±14.26	
7	70.24±10.12	0.7431	66.59±7.78	0.2207	44.30±17.93	0.6341	26.33±14.26	0.8693
14	67.39±9.41	0.7950	60.25±8.32	0.7742	55.88±16.54	0.3061	30.10±12.57	0.4891
21	64.61±7.84	0.3837	62.32±3.59	0.8382	24.70±11.75	0.0027**	18.00±5.44	0.1960

*means in the same column with asterisk were significant difference (P<0.05)

Table 3.5 : Effect of treatment of sheep naturally infected with GINs with albendazole and Ivermectin on alanine aminotransferase and aspartate aminotransferase level (N=8)

Parameters	Alanine aminotransferase (IU/L)				Aspartate aminotranserase (IU/L)			
Treatment	Albendazole		Ivermectin		Albendazole		Ivermectin	
Days	Mean±SD	P value	Mean±SD	P value	Mean±SD	P value	Mean±SD	P value
0	174.6±95.04		181.9±129.9		414.7±15.06		487.4±107.5	
7	477.2±252.17	0.0589	691.9±721.9	0.0697	154.20±114.2	0.0151*	140.4±101.7	0.0238*
14	124.7±40.16	0.2445	329.4±400.3	0.3407	480.20±11.54	0.3455	457.3±91.58	0.5720
21	145.5±58.20	0.6202	106.7±100.2	0.2627	357.5±162.6	0.4922	692.7±453.0	0.2329

*means in the same column with asterisk were significant difference (P<0.05)

Table 3.6: Effect of treatment with Albendazole and Ivermectin on urea concentration (mg/dl) of sheep naturally infected with gastrointestinal nematodes

Days	Albendazole		Ivermectin	
	Mean±SD	P value	Mean±SD	P value
0	39.14±22.07		47.60±17.93	
7	39.30±11.95	0.9860	53.89±27.11	0.5928
14	41.37±14.59	0.8145	56.93±23.99	0.3934
21	51.57±19.22	0.2495	60.63±25.09	0.2522

Table 3.7: Effect of treatment of sheep naturally infected with GINs with Albendazole and Ivermectin on calcium and phosphorus concentration

[parameter	Calcium (mg/dl)				Phosphorus (g/dl)			
Treatment	Albendazole		Ivermectin		Albendazole		Ivermectin	
Days	Mean±SD	P value	Mean±SD	P value	Mean±SD	P value	Mean±SD	P value
0	67.25±13.71		70.86±13.02		3.44±0.27		2.68±0.36	
7	83.63±27.85	0.1578	85.00±10.62	0.0235*	2.96±0.52	0.0363*	3.09±0.28	0.0235*
14	84.38±12.95	0.022*	89.00±19.32	0.0755	3.19±0.30	0.1117	3.09±0.46	0.0755
21	86.38±11.61	0.0093**	97.67±5.86	0.750	2.77±0.30	0.0006***	3.19±0.65	0.750

*means in the same column with asterisk were significant difference (P<0.05)

3.2 The second experiment:

3.2.1 Drug testing:

The two drugs: Tetramisole and Levamisole, used in the second experiment were within the acceptance limits of assay% by the Directorate of Veterinary Medicines Registration (NMPB), Sudan (Table 3.8).

3.2.2 Faecal egg count reduction test:

In the second experiment, neither of the two drugs resulted in any significant reduction (≥95%) of the overall numbers of eggs being released by the sheep. At day 14 Tetramisole showed only 62.8% while Levamisole exhibited 91% reduction in egg count (Table 3.9).

Table 3.8: Assay (%) of Tetramisole and Levamisole

Generic name/ Dosage form	Type	Average retention time	Area	Sample			
				Reference	Specification	Sample Content % (N=3)	RSD %
Tetramisole HCl oral solution	Standard	5.605	883674.3	Bash Pharma	90-110 %	98.0	0.537
	Sample	5.499	1057544				
Levamisole HCl oral solution	Standard	6.888	1730133	BP, 2016	92.5-107.5 %	103.2	0.181
	Sample	6.884	1723912				

Table 3.9: Mean faecal egg count (arithmetic) and reduction (%) for Tetramisole and Levamisole-treated sheep

Days	Tetramisole		Levamisole	
	epg (arithmetic mean)	Reduction %	epg (arithmetic mean)	Reduction %
0	3325	-	3325	-
2	250	92.5	450	86.5
3	475	85.7	462.5	86.1
4	1063	68	600	82
7	1225	63.2	762.5	77.1
10	962.5	71.1	612.5	81.6
14	1238	62.8	300	91
21	2013	39.5	1450	56.4

3.2.3 Safety of Levamisole and Tetramisole administration in sheep naturally infected with GINs

There was significant decrease in total protein concentration in animals treated with levamisole at day 7. The level decreased and increased at days 14 and 21 post treatment, respectively with no significant ($P>0.05$) change. In the second group (animals treated with tetramisole) there was no significant ($P>0.05$) in total proteins concentration during the entire period of the experiment (Table 3.10). As we could observe in Table (3.10), There was significant ($P<0.05$) decrease in albumin concentration in animals treated with levamisole at days 14 and 21 post treatment.

In Table (3.11) there was no significant change in the two enzymes evaluated in the current study in the animals treated with levamisole and Tetramisole. In the animals treated with tetramisole there is prominent, although none significant, increase in AST level at day 7 post treatment when compared with pre-treatment level.

There was no significant ($P<0.05$) difference in urea concentration between pre-treatment and post-treatment values in the two treatment groups (Table 3.12). Calcium exhibited no significant ($P>0.05$) increase in the two treatment groups following administration of levamisole and Tetramisole. There was significant ($P<0.05$) increase in phosphorus concentration in the animals treated with tetramisole at day 21 post treatment, while there was no significant ($P>0.05$) change in the animals treated with levamisole (Table 3.13).

There was significant decrease in total protein concentration in animals treated with levamisole at day 7. The level decreased and increased at days 14 and 21 post treatment, respectively with no significant ($P>0.05$) change. In the second group (animals treated with tetramisole) there was no significant ($P>0.05$) in total proteins

concentration during the entire period of the experiment (Table 3.10). As we could observe in Table (3.10), There was significant ($P<0.05$) decrease in albumin concentration in animals treated with levamisole at days 14 and 21 post treatment.

In Table (3.11) there was no significant change in the two enzymes evaluated in the current study in the animals treated with levamisole and Tetramisole. In the animals treated with tetramisole there was prominent, although none significant, increase in AST level at day 7 post treatment when compared with pre-treatment level.

There was no significant ($P<0.05$) difference in urea concentration between pre-treatment and post-treatment values in the two treatment groups (Table 3.12). Calcium exhibited no significant ($P>0.05$) increase in the two treatment groups following administration of levamisole and Tetramisole. There was significant ($P<0.05$) increase in phosphorus concentration in the animals treated with tetramisole at day 21 post treatment, while there was no significant ($P>0.05$) change in the animals treated with levamisole (Table 3.13).

Table 3.10: Effect of treatment of sheep naturally infected with GINS using Levamisole and Tetramisole on total proteins and albumin concentration

Parameter	Total proteins (g/L)				Albumin (g/L)			
Drugs	Levamisole		Tetramisole		Levamisole		Tetramisole	
Days	Mean±SD	P value	Mean±SD	P value	Mean±SD	P value	Mean±SD	P value
0	64.08±5.79		68.08±12.85		61.65±11.39		26.77±9.01	
7	56.18±7.27	0.0357*	64.82±7.16	0.5409	52.92±9.57	0.1192	29.67±7.92	0.5045
14	59.51±8.78	0.2492	66.03±7.77	0.7048	27.54±4.96	0.0001***	37.30±13.48	0.0874
21	71.91±10.18	0.0849	65.10±7.29	0.5778	39.31±15.24	0.0064**	27.20±4.31	0.9041

*means in the same column with asterisk were significant difference (P<0.05)

Table 3.11: Effect of treatment of sheep naturally infected with GINS using Levamisole and Tetramisole on ALT and AST level

Parameter	ALT (IU/L)				AST (IU/L)			
Drugs	Levamisole		Tetramisole		Levamisole		Tetramisole	
Days	Mean±SD	P value	Mean±SD	P value	Mean±SD	P value	Mean±SD	P value
0	NA		254.6±208.5		451.1±217.2		494.7±161.6	
7	NA		135.8±87.62	0.2174	406.7±180.9	0.6638	953.0±678.7	0.0843
14	NA		276.5±154.8	0.8156	324.3±189.7	0.2533	598.6±307.4	0.4182
21	NA		155.2±60.11	0.2834	415.7±197.2	0.7482	465.6±196.8	0.7513

*means in the same column with asterisk were significant difference (P<0.05)
NA= No available data

Table 3.12: Effect of treatment with Levamisole and Tetramisole on urea concentration (mg/dl) of sheep naturally infected with gastrointestinal nematodes

Days	Levamisole		Tetramisole	
	Mean±SD	P value	Mean±SD	P value
0	58.95±14.06		60.78±13.26	
7	49.00±21.84	0.2972	53.39±18.39	0.3724
14	51.55±16.95	0.3723	52.50±11.74	0.2069
21	55.18±26.13	0.7288	53.10±16.64	0.3241

Table 3.13: Effect of treatment of sheep naturally infected with GINS using Levamisole and Tetramisole on calcium and phosphorus concentration

Parameter	Calcium (mg/dl)				Phosphorus (g/dl)			
Drugs	Levamisole		Tetramisole		Levamisole		Tetramisole	
Days	Mean±SD	P value	Mean±SD	P value	Mean±SD	P value	Mean±SD	P value
0	78.13±11.18		78.13±22.04		2.95±0.63		3.07±0.49	
7	79.25±14.81	0.8663	76.00±12.46	0.8158	3.05±0.47	0.7096	3.33±0.66	0.3819
14	83.17±10.68	0.4117	91.13±23.15	0.2693	3.09±0.81	0.7161	3.32±0.55	0.3554
21	80.57±12.96	0.7008	84.50±3.89	0.4339	3.69±0.88	0.0800	4.10±0.95	0.0158*

*means in the same column with asterisk were significant difference (P<0.05)

Chapter Four

Discussion

The current study signifies the presence of multiple drug resistance for the three major anthelmintics groups' common in use in sheep in Sudan. Resistance has been characterized by Shoop *et al.*, (1995) who determined the resistance of whatever medicine by comparison the previously effectiveness status with 95% elimination of the target parasite and become less. Also resistance considers present if the percentage reduction in faecal egg count (FEC) after treatment was less than 95%, and the lower limit of the 95% confidence interval was less than 90%. If only one of the two criteria was met, resistance was suspected (SR) to be present (Coles *et al.* 1992, and Domke *et al.*, 2012).

The compatibility of the four medicines with the registration guidelines (NMPB, 2017), following chemical analysis supports accuracy of dosing and stability of drugs under investigation.

Here in the first experiment both albendazole and ivermectin failed to eliminate nematode helminths from sheep with average 33.8% and 48.5% reduction in faecal egg count, respectively. The development of benzimidazoles efficacy reduction has been reported worldwide, where it was first appeared in sheep in the UK (Britt, 1982) followed by similar report that documented resistance in Scottish sheep flocks (Bartley *et al.*, 2003). In Germany the first report was by Bauer, (2001). In Australia the initial one was in 1986 (Webb and Ottaway, 1986), followed by Waller *et al.*, (1995); and Love and Coles, (2002).

In Africa, a study in Ethiopia to estimate the efficacy of albendazole in goats by using the recommended sheep dose with different rates; revealed considerable change in the efficacy of albendazole in goats, while doubling and tripling the recommended dose (3.8, 5.7 and 7.6 mg/kg) gave the near results (65.5, 81.4 and 84.1%) respectively. On the

other hand, using the recommended dose in sheep showed reduction of efficacy which was 62% near to the result obtained in goats at the same dose level (Eguale *et al.*, 2009).

In south Africa the results of efficacy by using (FECRT) for albendazole, levamisole and closantel were over 80% in 12 farms, when was considered as the cut point for the efficacy reduction and most of the farms respected as effective with few cases out of the cut point (Bakunzi, 2008), in their study they used different rate of efficacy than that recommended by Coles *et al.*, (1992). Also in South Africa a combined survey has been conducted on 52 farms aimed to target *Haemonchus* spp. to estimate efficacy of four medicines (albendazole, levamisole, ivermectin and rafoxanide), < 60% susceptible to three of the four anthelmintics tested, and 8 % of the strains were < 40 % susceptible to all four of the anthelmintics (Van Wyk *et al.*, 1999)

Reduced efficacy of ivermectin obtained in the current study could be attributed to the continuous and indiscriminate use of that drug in the field and correlates with the justification that resistance development to ivermectin follows the excessive using in the field due to its wide spectrum activity against ecto-endo parasite's (Adediran and Uwalaka, 2015).

While many studies have demonstrated deterioration in the efficacy of levamisole in controlling of GIT parasites in goats (Yadav and Uppal, 1992; Yadav *et al.*, 1995 and Ram *et al.*, 2007), but Jaiswal *et al.*, (2013) indicated contradictory results, where levamisole is still maintained its efficacy, this maintaining could refer for the less regular applying of Levamisole. In the current study the situation for levamisole is much better when compared with albendazole, ivermectin and tetramisole with 91% reduction in epg count in sheep. The popularity of albendazole and ivermectin among sheep owners as effective, economic and easy to

administer anthelmintics justify the less regular application of levamisole in sheep health care management.

Considering efficacy <95% as cut point for resistance occurrence, tetramisole showed only 62.8% reduction in epg count, a result which could be justified by the mechanism of action of the drug that is similar to levamisole as both of them are cholinergic drugs. Levamisole, its activity at the neuromuscular junction (NMJ), as an agonist at nicotinic acetylcholine receptors (nAChR) result in a spastic paralysis at the level of nematode. Hence resistance to member of the group present; will spread to other and this is usually due to reduction of binding affinity site to the levamisole analogue (Sangster *et al.*, 1998, Wolstenholme *et al.*, 2004).

Multi- drug efficacy reduction has been reported worldwide, where in goat has been documented in 15 Danish goat herds by using faecal egg count reduction test (FECRT), egg hatch assay (EHA) and larval development assay (LDA), where 6 farms have revealed reduction for BZs and LEV, and for IVM and BZs on one farm (Maingi *et al.*, 1996b). Resistance to a particular medicine would transfer to other one of the same group as has been detected by El-Abdellati *et al*., (2010) when efficacy reduction to Moxidectin proved although it hasn't been used at this farm before, contrary to ivermectin where has been used.

The results of multi- efficacy reduction have been confirmed in goat (Jaiswal et al. 2013) for albendazole, levamisole and ivermectin had shown (53%, 65% and 76%) at day 14 post treatment (Gelot *et al.*, 2016), a result that supports the present findings.

Continuous and valuable follow up for the status of efficacy reduction must perform even in areas of sporadic cases of efficacy reduction (Dolinská *et al.*, 2014), where by tracing reports over world, the failure of anthelmintics medicines become a global issue extends to

Europe when most AR records were for benzimidazole- or levamisole-resistance and with descriptive cases of macrocyclic lactones resistance, intensely for ivermectin (Papadopoulos, 2008), Western Australia revealed multiple-resistance in *T. circumcincta* specifically to benzimidazole and levamisole (including *Trichostrongylus* spp.) has been detected by Besier, and Love, (2003). A remarkable importance in certain African countries such as south African *H. contortus* showed the highest level of resistance in the world that hasn't been recorded before to four types of anthelmintics medicines (albendazole, levamisole, ivermectin and rafoxanide) by using (FECRT) (Van Wyk *et al.*,1999), and Kenya that *H. contortus, Trichostrongylus, Oesophagostomum* spp. displayed multiple resistance to albendazole, levamisole, ivermectin and rafoxanide in Kenya by Waruiru *et al*. (1998).

In Argentina Entrocasso *et al.*, (2008) documented that inconsiderable value have been added with the combined anthelmintics treatment against GINs in comparison with un-combined, and the results were as follow: 73.4% (albendazole $_{IV}$), 79.0% (ivermectin $_{IV}$), 91.9% (albendazole $_{IV}$ + ivermectin $_{IV}$), 43.5% (albendazole $_{IR}$), 79.8% (ivermectin $_{SC}$) and 70.8% (albendazole $_{IR}$ + ivermectin $_{SC}$). The efficacy against *Haemonchus* spp. was 95.1 (albendazole $_{IV}$), 99.3 (IVM_{IV}) and 99.9% (albendazole $_{IV}$ + IVM $_{IV}$), while the efficacy against *Trichostrongylus colubriformis* for the same treatment groups was 79.6, 100 and 99.9% respectively.

Another study conducted in Nigeria in sheep GINs, using FECRT it revealed well efficacy of albendazole (99%), ivermectin (96%) and levamisole (96%), with lower 95% confidence interval (91,89 and 89) respectively, where it had shown efficacy reduction to ivermectin and levamisole and suspicious to albendazole (Adediran and Uwalaka, 2015).

There are many factors that encounter the animals to keep anthelmintics efficacy, could be concluded as follows: updated status of efficacy of currently marketed anthelmintics is a basic requirement and first barrier to encounter resistance particularly within regions where medicines are still effective (Dolinská *et al.*, 2014). Unfortunately, the absence of new medicines with different mechanism of action makes the AR challenge (Prichard, 2008), well management of the recent medicines is needed to keep efficacy of them (Coles *et al.*, 2006).

Strategies to combine medicines from the same or different anthelmintics groups to fortify and widen the spectrum; have also shown well advancing in efficacy reduction, as in New Zealand sheep's, where benzimidazoles (BZ) displayed in 60% (9/15) of the farms, to levamisole (LEV) in 66% of farms (10/15), combination drench (BZ+LEV) on 43% of farms (3/7) and avermectin on 1 of 8 farms (Sharma, 2004)**.** Also combination (ABZ + TET), in addition to compare with ABZ, TET, and IVM in nematode of sheep and goats, have demonstrated well efficacy in eastern Ethiopia (Sissay *et al.*, 2006), but other study some years later in Southern Ethiopia with the same medicines has defeated this (Sheferaw *et al*., 2013).

Melaku *et al.*, (2013) conducted a study in North Western Ethiopia, targeted ABZ, IVM, TET, LEV, ABZ, IVM+ABZ, ABZ+LEV in 28 naturally infected sheep, where reported excellent efficacy of combined medicines groups (IVM+ABZ and ABZ+LEV) (100%) and the least one was in TET group (89.51 %), and between scattered others groups; ABZ (99.08 %), IVM (96.69 %), LEV (90.06 %).

There was no significant change in total proteins concentration at day 7 in both treatment groups following administration of albendazole and ivermectin. The total proteins values reported in the current study for the pre-treatment level for albendazole group (68.62±9.20), and

ivermectin group (61.67±7.60) were within the range (60-79.0 g/l) recommended by Kaneko *et al.,* (1997) in sheep. The non-significant decrease in total proteins observed thereafter in the current study may be attributed to the helminths infection. There was sharp significant ($P<0.05$) decrease in albumin concentration in animals treated with albendazole at day 21 following treatment. The same reduction in albumin concentration was also observed in ivermectin treated-group. Kaneko *et al.,* (1997) reported a normal range for albumin as: 24-30 g/l; this decrease is justified by the failure of treatment to eliminate parasitic infection. No available reports indicated significant effect of treatment on total protein and albumin concentration. Administration of abamectin to health sheep induced no significant decrease in total proteins and no significant increase in albumin concentration (Kužner *et al.*, 2005) while doramectin induced minor non-significant decrease in total protein and significant decrease in albumin concentration in sheep (Kužner *et al.*, 2005).

There was prominent increase (although non-significant) in the level of ALT enzyme at day 7 post treatment in the two animal groups. The level was almost the same to that of the pre-treatment values at days 14 and 21 post treatment. Kaneko *et al.,* (1997) indicated that the normal range for ALT level in sheep is: 30±4. Kužner *et al.*, (2005) indicated no significant decrease in ALT concentration following treatment with either abamectin or doramectin. There was significant ($p<0.05$) decrease in AST level in the two treatment groups (albendazole and ivermectin) at day 7 post treatment. The level of the enzyme returned to almost the same level at pre-treatment day with no significant difference with day zero. The normal level of AST in sheep is: 60-280 U/L (Kaneko *et al.*, 1997). The level of the enzyme is still within the recommended range. The increase in both enzymes observed at day 7 following treatment may be attributed to enzyme induction.

There was no significant change in urea concentration in the two treatment groups following administration of albendazole and ivermectin. Kaneko and his colleagues (1997) suggested that the normal range of urea in sheep is: 8-20 mg/dl (2.86-7.14 mmol/L). Urea values obtained in the current study were high when compared with the normal range suggested by Kaneko *et al.,* (1997). Calcium concentration increased significantly ($P<0.05$) following treatment with albendazole at days 14 and 21. In the same line there was significant increase ($P<0.05$) in calcium concentration following treatment with ivermectin at days 7 and 21 following treatment. The normal range of calcium in sheep is: 2.88-3.20 mmol/L (11.5-12.8 mg/dl) (Kaneko *et al.,* 1997). Here still the level of calcium is below that range recommended by Kaneko *et al.,* (1997). Administration of abamectin to health sheep resulted in non-significant increase in calcium level (Kužner *et al.,* 2005). Doramectin administration induced significant reduction in calcium level at days 15 and day 42 following treatment (Kužner *et al.,* 2005).

There was significant ($P<0.05$) decrease in phosphorus concentration at days 7, and 21 in the animals treated with albendazole. While in the animals treated with ivermectin there was significant ($P<0.05$) increase in phosphorus concentration at day 7 post treatment. The normal range of phosphorus in sheep is: 1.62-2.36 mmol/L (5.0-7.3 mg/dl) (Kaneko *et al.,* 1997). The level of phosphorus in the blood of the treated sheep was low when compared with the range suggested by Kaneko *et al.,* (1997).

Only limited data were available for the biochemical safety of the drugs under investigation. Ivermectin given in three times the recommended dose did not cause adverse effects in sheep (Shoop and Soll, 2002). Doramectin did not cause toxic effects even after treatment with 1.5 mg/kg b.w. 97.5X the recommended dose) (Conder and Baker,

2002). Kužner and his colleagues (2005), concluded that administration of abamectin and doramectin to sheep at recommended dose 0.2 mg/kg b.w. induced some significant differences in some haemtological and biochemical parameters when the control and treated animals. But still the blood metabolites are within the normal range. No neurological symptoms were observed, and hence abamectin and doramectin might be well tolerated in sheep (Kužner *et al.*, 2005).

There was significant decrease in total protein concentration in animals treated with levamisole at day 7. The level increased at days 14 and 21 post treatment, respectively with no significant ($P>0.05$) change. In the second group (animals treated with tetramisole) there was no significant ($P>0.05$) change in total proteins concentration during the entire period of the experiment. Still the values are within the range recommended by Kaneko *et al.,* (1997). There is significant ($P<0.05$) decrease in albumin concentration in animals treated with levamisole at days 14 and 21 post treatment. Still the level did not exceed the recommended range.

There was no significant change in the two enzymes evaluated in the current study in the animals treated with Levamisole and Tetramisole. In the animals treated with tetramisole there is prominent, although none significant, increase in AST level at day 7 post treatment when compared with pre-treatment level. This may be attributed to enzyme induction; where the metabolizing enzymes are increased immediately following administration of drugs.

There was no significant ($P<0.05$) difference in urea concentration between pre-treatment and post-treatment values in the two treatment groups. Although, the level is high when compared with the normal range recommended by Kaneko *et al.,* (1997). Calcium exhibited no significant ($P>0.05$) increase in the two treatment groups following administration of

Levamisole and Tetramisole. There was significant ($P<0.05$) increase in phosphorus concentration in the animals treated with tetramisole at day 21 post treatment, while there was no significant ($P>0.05$) change in the animals treated with Levamisole. The level is still within the recommended range for phosphorus in sheep (Kaneko *et al.*, 1997).

As the fluctuation in the level of blood metabolites observed in the current experiments did not exceed the normal range recommended for sheep (Kaneko *et al.*, 1997). The four drugs are to be considered safe to be administered to sheep at the recommended dose level.

It is concluded that therapeutic doses of Albendazole, Ivermectin, Levamisole and Tetramisole do not induce clinically important adverse reactions in sheep. Therefore, they can be used in sheep following the manufacturers recommended dose regime.

Conclusion and Recommendations

Conclusion

All the four used medicines that descended from three groups had shown advanced status of efficacy reduction, which are less than recommended percent by Coles *et al.*, (2006) where medicines should have a minimum ability to reduce the percentage till 95% with arithmetic means ≥90.

Recommendations

1. Further studies using in vitro methods as well as molecular techniques to characterize anthelmintics resistance up to the genus level are required.
2. Alternative drugs not common in use in Sudan such as Moxidectin, Doramectin and Abamectin may be used to overcome the problem.
3. Newly discovered drugs, such as monepantel and derquantel should be evaluated under field conditions, before their introduction in the veterinary health care system.

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