Evaluation of Anthelmintic Properties of Ethanolic Extracts of Leaf of River Red Gum *(Eucalyptus camaldulensis*) using Barber Pole Worm (*Haemonchus contortus)* on Yankasa Sheep

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Abstract

It is estimated over 90 % of endoparasites cases in small ruminants are due to such as Haemonchus contortus, which causes, anemia, diarrhoea, in severe cases, death. Currently a lot of chemicals is being used for control in some parts of the world. However, reports of resistant nematodes and drugs residues are of concern. Thus, aim of this study was to evaluate the Anthelmintic Properties of Ethanolic Extracts of Leaf of River Red Gum (Eucalyptus camaldulensis) using Barber Pole worm (Haemonchus contortus) as test worms. Twenty five Yankasa breed of Sheep were experimentally infected with Barber pole worm for three months were randomly allotted to five treatments (T1, T2, T3, T4 and T5) with five replicates adopting Complete Randomized Design (C R D) where T1 - distilled water (negative control), T2 - Albendazole (positive control) at 25 mg/kg body weight, T3 - 100 mg/kg body weight, T4 - 400 mg/kg body weight and T5 - 800, mg/kg body weight of leaf extracts. A preliminary phytochemical screening of the ethanolic extract of leaf of River Red Gum revealed the presence of Alkaloids, Saponins, Tannins, Resin, Flavonoids, and Anthraquinone. Feacal Eggs Count Reduction Test (FECRT) were studied and the activity revealed in - vivo dose - dependant reduction egg per gram (epg) of 30, 81, 95 % , of the graded extracts respectively while albendazole (positive control) reduced 98 %, at 14 days post treatment. Acute oral toxicity test revealed LD_{50} *in mice at 800 mg/ kg body weight. No significant ($p > 0.05$) difference were observed in egg per gram count (epg) between control and graded doses of ethanolic leaf of River Red Gum (Eucalyptus camaldulensis). Also, no toxic effects were noticed during toxicity and clinical examination of the mice and sheep respectively. The obtained results suggest strong anthelmintic properties of extract of leaf of River Red Gum (Eucalytus camaldulensis) against Barber Pole worm (Haemonchus contortus) probably due to stated phytochemicals, thus support the use as safe, for Sheep at dose of 800 mg/kg body weight. Therefore, the leaf extract has the potential for anthelmintic*

Keyword: Anthelmintic resistance, Drugs residues, EPG, FECRT, sheep.

Évaluation des propriétés anthelminthiques des extraits éthanoliques de feuille de gomme rouge de rivière (*Eucalyptus camaldulensis*) à l'aide du ver Barber Pole (*Haemonchus contortus*) sur le mouton Yankasa

Résumé

On estime que plus de 90 % des cas d'endoparasitisme chez les petits ruminants sont dus à Haemonchus contortus, qui provoque de l'anémie, de la diarrhée et, dans les cas graves, la mort.

Actuellement, de nombreux produits chimiques sont utilisés à des fins de contrôle dans certaines régions du monde. Cependant, les rapports faisant état de nématodes résistants et de résidus de médicaments sont préoccupants. Ainsi, le but de cette étude était d'évaluer les propriétés anthelminthiques des extraits éthanoliques de feuilles de gomme rouge de rivière (Eucalytus camaldulensis) en utilisant le ver Barber Pole (Haemonchus contortus) comme vers tests. Vingt-cinq moutons de race Yankasa ont été infectés expérimentalement par le ver Barber pendant trois mois et ont été répartis au hasard entre cinq traitements (T1, T2, T3, T4 et T5), avec cinq répétitions adoptant un plan randomisé complet (PRC) où T1 - eau distillée (négatif). contrôle), T2 - Albendazole (témoin positif) à 25 mg/kg de poids corporel, T3 - 100 mg/kg de poids corporel, T4 - 400 mg/kg de poids corporel et T5 - 800 mg/kg de poids corporel d'extraits de feuilles. Un examen phytochimique préliminaire de l'extrait éthanolique de feuille de River Red Gum a révélé la présence d'alcaloïdes, de saponines, de tanins, de résine, de flavonoïdes et d'anthraquinone. Le test de réduction du nombre d'œufs fécaux (TRNŒF) a été étudié et l'activité a révélé une réduction in vivo des œufs par gramme (epg) en fonction de la dose de 30, 81, 95 %, des extraits classés respectivement, tandis que l'albendazole (témoin positif) réduisait 98 %, à 14 jours après le traitement. Un test de toxicité orale aiguë a révélé une DL50 chez la souris à 800 mg/kg de poids corporel. Aucune différence significative ($p > 0,05$) n'a été observée dans le nombre d'œufs par gramme (epg) entre les doses témoins et les doses graduées de feuille éthanolique de River Red Gum (Eucalyptus camaldulensis). De plus, aucun effet toxique n'a été observé lors de l'examen toxicologique et clinique des souris et des moutons respectivement. Les résultats obtenus suggèrent de fortes propriétés anthelminthiques de l'extrait de feuille de River Red Gum (Eucalytus camaldulensis) contre le ver Barber Pole (Haemonchus contortus), probablement dues aux composés phytochimiques indiqués, soutiennent donc l'utilisation comme sûre, pour les moutons à la dose de 800 mg/kg corporel. poids. Par conséquent, l'extrait de feuille a un potentiel anthelminthique.

Mots-clés : Résistance aux anthelminthiques, Résidus de médicaments, EPG, TRVŒF, mouton.

تشير التقديرات إلى أن أكثر من تسعين في المائة من حالات التقيح الداخلي في المجترات الصغيرة ترجع إلى مثل Haemonchus contortus التي تسبب فقر الدم والإسهال وفي الحالات الشديدة تؤدي إلى الوفاة، يتم حاليًا استخدام الكثير من المواد الكيميائية للتحكم فيها في بعض أجزاء العالم ومع ذلك، فإن التقارير عن مخلفات النيماتودا والعقاقير المقاومة هي موضع الاهتمام، وبالتالي، كان الهدف من هذه الدراسة هو تقييم الخصائص الأنثلمينية لمستخلصات إيثانوليك من أوراق نهر الصمغ الأحمر باستخدام دودة القطب الحلاق (Haemonchus contortus) كديدان اختبار. تم تخصيص خمسة وخمسون سلالة من الأغنام من يانكاسا بشكل تجريبي بدودة عمود الحلاق لمدة ثلاثة أشهر بشكل عشوائي لخمسة علاجات (T1, T2, T3, T4 وT5) مع خمسة نسخ مكررة تعتمد التصميم العشوائي الكامل الأولالمياه المقطرة (التحكم السلبي) والثاني البيندازول (التحكم الإيجابي) بوزن 25 مليغرام/كيلوغرام من وزن الجسم، والثالث100 مليغرام/كيلوغرام وزن الجسم، والرابع 400 مليغرام/كيلوغرام والخامس ٨٠٠ مليغرام/كيلوغرام من مستخلصات الأوراق كشف الفحص الكيميائي النباتي الأولي للمستخلص الإيثانوليكي لورقة نهر الصمغ الأحمر عن وجود القلويدات والسابونينات والعفص والراتنج والفلافونويد والأنثراكينون تمت دراسة اختبار تقليل عدد بيض الجنين تم الكشف عن نشاط في - جرعة حية - البيضة المعتمدة على التخفيض لكل جرام من 30، 81، 95 %، من المستخلصات المتدرجة على التوالي بينما خفض الألبيندازول (التحكم الإيجابي) 98 %، بعد أربعة عشر يومًا من العلاج كشف اختبار السمية الفموية الحادة عن الجرعة المميتة 50 في الفئران بوزن 800 مجم/كجم من وزن الجسم لم يلاحظ أي فرق كبير ($p > 0.05$) في عدد البيض لكل جرام بين جرعات التحكم والجرعات المتدرجة من الورقة الإيثانولية لنهر الصمغ الأحمر أيضًا، لم يلاحظ أي تأثيرات سامة أثناء السمية والفحص السريري للفئران والأغنام على التوالي تشير النتائج التي تم الحصول عليها إلى خصائص خلدية قوية لاستخلاص أوراق نهر الصمغ الأحمر ضد دودة القطب الحلاق ربما بسبب المواد الكيميائية النباتية المذكورة، وبالتالي دعم الاستخدام كآمن، للأغنام بجرعة 800 مليغرام/كلوغرام من وزن الجسم. لذلك، فإن مستخلص الأوراق لديه القدرة على التلدين.

Introduction

Sheep production plays a critical role in supporting socio-economic status of the rural masses, particularly the small landholders and landless farmers, who rely on these animals for their animal protein source and income for their livelihood (Lateef, 2003). They have been estimated to provide up to 30 % of the meat and 15 % of the milk supplies in sub-Saharan Africa, which thrive in a wide range of ecological regions often in conditions too harsh for rearing of cattle.

In sheep, parasitic diseases, especially gastrointestinal (GI) helminths, of primary concern is the Barber Pole worm (*Haemonchus contortus*) the Barber Pole worm is prevalent on farms and is among the factors that limit sheep production worldwide, accounting for the largest economic losses due to retarded growth, weight loss, reduced feed intake, lower milk production, impaired fertility, and in cases of massive infections and, high mortality rates (Cavalcante *et al.*, 2009). The worm has become increasingly resistant to anthelmintic drugs that are available. Generally, worms that develop resistance to one drug can no longer be killed by any member of the group to which that drug belong. And also, problem of drug residues in animal products.

Currently, nematode control programmes in sheep depend mainly on the use of anthelmintics. Development of anthelmintic resistance (Taylor *et al.*,2009), increased public awareness over the drug residues in animal products and toxicity problems (Muhammad *et al.*,2004) has necessitated an intensified effort to find alternative endoparasite control measures that are both feasible and accessible to the farmers (Ademola *et al.*,2010). Among the alternative strategies, is considerable and expanding interest in traditional herbal dewormers in both developed and developing countries. Several studies have shown that plant species can effectively reduce the degree of parasite infestation in livestock and are promising alternatives to conventional anthelmintics (Eguale *et al.*, 2011; Jorge *et al.*, 2011) that are both sustainable and environmentally acceptable.

Plants produce secondary metabolites that can inhibit protozoan, bacteria, fungi, viruses and pests. Extraction of their physiological activity also facilitates pharmacology studies leading to synthesis of a more potent drug with reduced toxicity (Ebana *et al*., 1991; Williams, 1996: Pamplona-Roger, 1999; Manna and Abalaka. These complementary components give the plants a safety and much efficiency.

In this context, *Eucalyptus camaldulensis* is an evergreen tree that has been widely used in traditional medicine for the treatment of various health disorders. *Eucalyptus* is the most important genera in the botanical family Myrtaceae; it is widely distributed in different regions around the world, with more than 800 species (Hassine *et al*., 2013). *Eucalyptus camaldulensis*, commonly known as the river red gum, is endemic in Australia (Singab *et al*., 2011). The plant has synonyms in other Nigerian languages, as Hausa call it – Itacenturari, Ngas in central Nigeria call it Tingkum. Leaf of *E. camaldulensis* are known to possess several biological and pharmacological activities, including antioxidant (Singab *et al*., 2011), cytotoxic (Singab *et al.*, 2011; Daniela *et al.,* 2007; Meshkani *et al.*, 2014), antimicrobial (Ghalem and Mohamed, 2008), larvicidal (Medhi *et al.*, 2010), pesticidal (Batish *et al*. 2008), and anti-dermatophytes (Falahati *et*

al., 2005). Literature surveys revealed the isolation and identification of some chemical ingredients from different parts of *E. camaldulensis* including eucalyptanoic acid (Sabira *et al.*, 2000), flavonoids (Abd-Alla *et al.*, 1980), acylatedpentacyclictriterpenoids (Siddiqui *et al*., 1997), and essential oils (Ghalem and Mohamed 2014; Gakuubi, 2016). The anthelmintics resistance to the existing chemical anthelmintic drugs is well known, so it is very important to search for alternative anthelmintics agents from natural source like plants or herbs to overcome this challenge (Ghareeb *et al.,* 2015).

In this scenario, how can the farmers know the effectiveness of the plant anthelmintic? It is essential to test the active substances beforehand with greater than 95% efficacy (Coles *et al*., 2006). The (FECRT) is used to determine the efficacy. This test consists of counting the fecal egg of the animals before and after treatment, and it is recommended to determine nematodes resistance or susceptible to the evaluated Product (Coles and Roush, 1992). This study is aimed to evaluate the anthelmintic efficacy of ethanolic leaf extract of leaf of *E.camaldulensis* to promote animal health and production, through the practice of ethno veterinary medicine.

Materials and methods

Experimental Site

The study was carried out at Teaching and Research Farm of Agric Science Department Livestock Unit of the Federal college of Education Pankshin Plateau state. Pankshin is situated in north central zone of Nigeria with latitude of 7°.43´N and longitude8°.53 N (Micro soft Encarta, 2008). Pankshin lies within the Guinea savannah region of Nigeria and hast wodstinct seasons; the wet season which lasts from April to October with an annual rain fall that ranges from1105 mm to 1600 mm and the dry season which lasts from November– March. The area is cool with an annual temperature range of 16.8 °C to 40.0°C and annual relative humidity which ranges between 39.5 ±220 % and 64.00 ±4 .8 % (TAC,2009).

Collection and Preparation of Crude Ethanolic extract

Fresh leaves of *Eucalyptus camaldulensis* were collected from the premises of Federal College of Education Pankshin plateau state. The leaves were rinsed under running tap water to remove any pollutants. After which the leaves were air dried under room temperature. The dried leaves were hammered with pistol and mortar and weighed (Gilani *et al*., 2004). Hundred (100 g) was poured into 700 ml of 100 % ethanol in conical flask. The mouth of the conical flask was covered with aluminium foil and kept for defined period of 72 hours with time to time shaking until it was thoroughly mixed and for complete elucidation of the active materials (Amin *et al.*, 2009). The dissolved extract was filtered through a fine cloth and final filtration was done through what man No.1 filter paper (Hussain *et al*., 2010). The filtered extract was poured into an open silver tray and fan until the filtrate was allowed to dry up and the filtrate placed in desiccator (Amin *et al.*, 2009). Finally, the residues (extracts) were used for the experiment.

Phytochemical screening of leaf of ethanolic Eucalyptus camaldulensis

The ethanolic leaf extract was subjected to a standard phytochemicals screening for major constituents including Saponin, Glycosides, Tannins, Flavonoids, Resins, Alkanoids, Amthraquinone and steroids using the method by Trease and Evans (1989).

Acute toxicity test

The median lethal dose, LD_{50} value of ethanolic leaf of *Eucalyptus camaldulensis* was determined using twelve healthy albino rats weighing an average of 180 g, according to the method of Lorke (1983). They were sourced from Vom Plateau state. The albino rats were kept in well-ventilated cages cushion with wood shavings. The rats were allowed to acclimatised to the experimental conditions for seven days during which they were fed with pelleted vital feed, and water provided *ad libitum*. The animals were randomly divided into four groups, each group having three rats and graded doses of the extract was distributed accordingly. Group A. 100 mg/kg body weight, Group B. 400 mg/kg body weight, group C. 800 mg/kg body weight, and group D. Distilled water. The acute toxicity test was in accordance with the organization for Economic Cooperation and Development guidelines (OECD, 2008). The ethanolic extract of leaf *Eucalyptus camaldulensis* were administered orally in single dose progression. Each Animal was carefully evaluated for 48h prior to deciding the dose to be given the next Animal. The monitored parameters included properties of skin and fur, eyes, respiratory patterns, autonomic nervous system, features such as salivation, diarrheoa, and ruin action, central nervous system features such as tremors, change in the level of activity, gait, and posture, and any other abnormal behaviour (Chandra *et al*., 2012).

Experimental design

A total of 25 Yankasa breed sheep with an average weight of 25 kg were bought at Pankshin livestock market Plateau state and was used for the research work. The sheep were free of any anthelmintic treatment for three months before trial. They were further divided randomly into five treatment groups with five replicates per treatment. Two weeks to the arrival of the animals, the experimental stalls were thoroughly washed and disinfected, feeding and watering troughs was washed and allowed to dry. On arrival of the animals, they were ear tagged for individual identification. undergone a prophylactic treatment against parasites and diseases as outline below:

Oxytetracycline Dihydrate L. A. injection (antibiotic) was administered 1ml/ 10 kg live body weight intramuscularly. Ivermectin injection was given. (anti-helminthic) was given 1 ml/50 kg live body weight. Diminazene Diaceturate injection (against Trypanosomiasis) was administered intramuscularly (I/m) at 0.5/10 kg live body weight. *V. Multinor*, a multivitamin was administered subcutaneously at 1ml/10kg live body weight. Pour on (against ectoparastite) was applied homogeneously and directly on the back of the animals. (Spine from the neck to the tail). The anthelmintic drugs were given prior to the beginning of the experiment to clear other gastrointestinal nematodes.

The animals were experimentally infested using Barber Pole worm (*Haemonchus contortus)* with an average heavy worm burden of infestation (Hansen and Perry 1994). The Experimental Design used was Complete Randomised Design (CRD). (T_1, T_2, T_3 T_4 T_5) T_1 distilled water (negative control), T_2 - Albendazole (positive control) 25 mg/kg body weight based upon the stated concentration and recommendations of the anthelmintic treatment. T_3, T_4, T_5, were orally, given ethanolic extract of leaf of *Eucalyptus camaldulensis* at graded doses of 100 mg/kg body weight, 400 mg/kg body weight, 800 mg/kg body weight for three consecutive days respectively. The animals were allowed to

graze freely on pasture with water *ad libitum*, for 90 days before the administration of the ethanolic extract *of* leaf of *E camaldulensis* was given, the animals were fasted and remained fasted until 24 hrs., after treatment and were allowed to graze. Feacal Samples were collected fourteen days to the end of experiment, day 0 and 14 days (pre/post) treatment to estimate Fecal eggs reduction count.

Collection and inoculation of experimental Test organism (Haemonchus contortus)

The test organism employed in this study were obtained from Department of Parasitology of National Vetenary Research Institute Vom Plateau State Nigeria. The Animals were experimentally infested in a single dose pattern according to Dobson *et al.,*1992).

Faecal Egg Count Reduction Test

A faecal Egg count reduction Test (FECRT) was used to evaluate the most effective dose extract as safe anthelmintic. 5 g faecal samples were collected directly from the rectums of all the sheep in each experimental group fourteen days to the end of the experiment days 0 and 14 (pre/post) treatments for epg count estimation, based on Ueno and Goncalves (1998).

Statistical Analysis

The reduction in Fecal Eggs count reduction test (FECRT) was calculated using the following formula FECRT = (T2/T1×C1/C2) ×100(?)

Whereby T1 was EPG before treatment (D0) in the Albendazole treatment group, T2 was EPG after treatment(D14) in albendazole treatment group, C1 is EPG before treatment in *Eucalyptus camaldulensis* leaf extract group (C-), and C2 is EPG after treatment in the *Eucalyptus camaldulensis* leaf extract treatment group (C-).

Data collected were subjected to one way analysis of variance (ANOVA) using SPSS version 27 Statistical Software, where significant differences occurred, the means were separated with same Statistical Software. All statements of significance were based on the 0.05 level of probability.

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Statistical Model

$Y_{ij} = \mu + T_i + \varepsilon_{ij}$

Y_{ij} = Individual Observation

μ = Overall mean

T_i = effects of graded doses *E.comuldulensis* (i^{th} treatment)

ε_{ij} = Experimental error containing all uncontrolled sources of variation

Results

Phytochemical Screening of Ethanolic Leaf Extracts of River Red Gum (Eucalyptus camaldulensis)

Table 1 Chemical constituents of River Red Gum (*Eucalyptus camaldulensis*) Leaves Extract

Phytochemical	Eucalyptus Camaldulensis (Red Gum)
Saponin	++
Tannin	+++
Flavonoid	+
Alkaloid	+
Glycosides	+
Resin	+++
Anthraquinone	+++

Key: + mildly presents; ++, highly present; +++, more highly present; -

The screening revealed the presence of Saponin, Glycosides, Tannins, Flavonoid, Resin, Alkaloids, Resin, and Anthraquinone, using method described by Lorke (1983) (Table 1) Anthraquinone, Resin and Tannins were the most abundant constituents follows by Glycosides, Saponins, Flavonoids Alkaloids.

Effect of Ethanolic Leaf Extract of (Eucalyptus camaldulensis) on Acute Toxicity Test on Albino Rat

Cage Side Observation

At dose < 800 mg/kg the albino rats look weak and dull for 24 - 48 h after which the animals normalize for the remaining 5 days. Other treatment (100 g/kg, 400 mg/kg) groups recorded normal behaviour, motor and neuronal functions for all the administered ethanolic leaf extracts with no mortality observed. The monitoring of skin and fur, eyes, behavioral pattern such as gait and posture, and autonomic and central nervous system activities of the rats remained unchanged when compared with distilled water (negative control) treatment during observation period of seven days.

Feacal Egg Count Reduction Test in Vivo Trial

Table 2: EPGs (mea n ± standard deviation) of Barber Pole Worm (*Haemonchus contortus*) and Efficacy (Reduction %) Control and Graded Dose Ethanolic Extract of Leaf of *Eucalyptus camaldulensis* on Feacal Egg Count Reduction Test

Treatment	Day 0	Day 14	Efficacy Reduction %	P - value	LOS
Distilled water (control -)	2140 ± 41.83	2280 ± 57.0	---	0.706	ns
Albendazole 25mg/kp (control +)	2160 ± 96.2	20 ± 44.7	98	0.563	ns
100mg/kg	2090 ± 89.4	1359 ± 51.9	30	0.643	ns
400mg/kg	2140 ± 74.2	482 ± 38.9	81	0.625	ns
800mg/kg	2110 ± 82.2	30 ± 44.7	95	0.501	ns

Key: *M±S.E, *= significant at p<0.05, ns= not significant, LOS=Level of Signifance, T_1= **Distilled Water (negative control)**, T_2= **(Albendazol) as positive control**, T_3= **200**

mg/kg of *ethanolic leaf extract, of* ***eucalyptus camaldulensis*** *T_4=* ***400 mg/kg of*** *ethanolic leaf extract, of* ***eucalyptus camaldulensis****, T_4=* ***800 mg/kg of*** *ethanolic leaf extract, of* ***eucalyptus camaldulensis.***

The Feacal Eggs Reduction Test (FECRT) results of the Barber Pole worm (*Haemonchus contortus*) burden in, infested sheep at day 0 and 14 after treatments are as shown in Table 2. A noticeable increase in EPG count in distilled water (negative control) from 2140 ± 41.83 at day 0 to 2280 ± 57.0 was observed at 14 days post treatment, while (albendazole) positive control decreased epg count from 2160 ± 96.2 at day 0 to 20 ± 44.7 at day 14 post treatment (98 ?), 100 mg/kg decreased in epg count from 2090 ± 89.4 at day 0 to 1359 ± 51.9 at day 14 post treatment (30 ?), 400 mg/kg decreased epg count from 2140±74.2 at day 0 to 482± 38.9 at day 14 post treatment (81 ?), 800 mg/kg decreased epg count from 2110±82.2 at day 0 to 30±44.7 at day 14 post treatment (95 ?). Sheep in albendazole positive control had a higher parasitemia count compared with the graded dose leaf extracts. There was no significant ($p > 0.05$) difference in epg between the graded doses of ethanolic leave extracts and the positive control group at day 0 and 14 post treatments.

Discussion

The present study investigated the anthelmintic potential of *E. camaldulensis.* Preliminary phytochemicals screening of the ethanolic extract of leaf of *Eucalyptus camaldulensis* revealed abundance of various qualitative and quantitative phytochemicals (Table 1) in the plant extracts, supporting its medicinal value against several diseases (Iwalewa *et al.,* 2003; Mohammed pours *et al.*, 2013; Orororo *et al*., 2010; Getahum., 2016). Detection of tannins, alkaloids, and other compounds strengthen its medicinal value (Iwalewa *et al*., 2011) This finding agreed with the work of Chuku *et al.*, (2016) that crude methanol leaf extracts of the plant contained moderate to high amount secondary metabolites: Alkaloids, saponins, tannins, flavonoids, Steroids, carbohydrate, cardiac glycosides, and Anthraquinone. On the other hand (Jouki and Khazaei 2010) reported that tannins, flavonoids, alkaloids and glycosides were not detected but saponins, volatile oils and balsam (Gum) where seen in leaf extracts of *E. camaldulensis* from Iran. Leaf extracts of *E.camaldulensis* are generally rich in tannins which vary in qualitative and quantitative values according to the origin of the sample and consequently protoanthocyanide level were influenced by the geographical origin (Cadahia *et al.,*1997).

Acute Toxicity Test on Albino Rats

The experimental mice used in this present study were orally given the plant extracts with single dose to each of the treatment groups T1 - 100, T2 - 400, and T3 - 800 mg/kg body weight respectively. Toxicity and mortality were not recorded within 24 h and subsequently for 7 days. Physical signs of toxicity, such as paw licking, salivation, stretching, and weakness were not recorded during the study. This finding is agreement with that of Islam *et at*., (2014) that reported LD_{50} high dose value of 1120 mg/kg after using the bark of methanolic extract of *E. camaldulensis* on Swiss albino mice (*Musmusculus*), indicating its low host toxic effects. Similarly (Blumenthal and Busse, 1998) reported safe daily oral dose in human adult as 300 - 600 mg/ kg body weight of the plant extracts. This present work does not agree with the work reported by Cheek (1998) that overdose with the plant extracts could cause gastrointestinal

burning, abnormal pain, vomiting and convulsions, depress respiration and the central nervous system. Even though his research is centre on humans, and did not state the LD_{50}, but the ones carried out on rats does not show any adverse effects on them, thus the findings from this work established estimated LD_{50} to be? >? 800 mg/kg body weight. Absence of mortality in the test animals indicated that the extracts did not result in acute toxicity (Salawu *et al*., 2009). Results obtained from this acute toxicity study pave way for the dose determination in the experimental work, and aid in the determination of LD_{50} value which provided many indices for future potential type of the anthelmintic drug activity.

Feacal Egg Count Reduction Test - in vivo trail

The results of the Feacal Analysis obtained at 14-day post treatment, revealed maximum reduction level of positive control (albendazole) treated group (98 ?) with only a single dose, which was observed to be same with the work of keyyu *et al.,* (2002) who found efficacy of 98%. The group that was treated with higher dose of 800 mg/kg body weight, (Table 2) indicated that the plant extracts eliminated the worms with (95 %) at 14 days post treatment which shows similar efficacy to that of conventional anthelmintic (Albendazole at 25 mg/kg body weight). At a dose of 400 mg/kg body weight, it revealed (81%) epg reduction and at a dose of 100 mg/kg body weight, it showed (30%) epg reduction count test. These findings established that ethanolic extract of leaf *of E.camaldulensis were* found to possess anthelmintic activities in in-vivo , in dose - dependent manner against Barber Pole worms (*Haemonchus contortus.)* It was observed to be more potent in the concentration of 800 and 400 mg/kg body weight and such prompt decrease in the parasites number is a testament that the ethanolic leaf extract of *E.camaldulensis* kill the parasites efficiently, but by an unknown mechanism. This finding is in agreement with the work reported by chalchat *et al.*, (1995) that the plant was observed to possess antitrypanasomal activities in dose - dependent manner in an acute form. Also in agreement with the present work is the report by Bennet-Jenkins and Bryant (1996) that 5 sheep were experimentally infected by *Haemonchus contortus* and fed with the plant leaf for 7 days showed 91? EPG reduction count. Furthermore, it was also reported by Joshi *et al.,* (1996) and Paveesh *et al.,* (1996) that extracts obtained from the plant was effective in mastitis prevention, treatment for endometritis and as an anthelmintic agent (dewormer). The activities of ethanolic leaf extract of E. *camaldulensis* could be due to the hydrophobic nature of the cyclic hydrocarbons, which allows the leaf extract of *E. camaldulensis* interact with the protozoans causing conformation changes in the parasites membrane structure, resulting in the loss of membrane stability (Calsamiglia *et al.,* 2007). The plant extracts also act by inhibiting some key enzymes in the parasites glycolytic pathway (Meciel *et al*. 2010) its effectiveness could also be as a result of combine effects of the active principles found in the ethanolic leaf extracts of *E. camalduensis* which agree with the work reported by Chalchat, *et al.*, (1995), that the plant contains Tannins as much as 11% and it's attributed to several activities including antidysenteric, antimutagenic, anthelmintic, bactericidal, cancer - preventive, antinephritic, antioxidant, antiviral, hepatoprotective, pesticide, psychotropic and virucidal properties of leaf ethanolic extracts of *E. camaldulensis* which is being exploited to mitigate against

diseases and ailments. This multicomponent nature of the leaf extracts is an advantage for several target sites on the worms which is of great importance, considering the fact that present chemotherapy consists of antimony compounds which are expensive, toxic and drug resistance is prevalent (Meciel *et al*. 2010) Leaf extracts of *E. camaldulensis* derivatives represent safe, inexpensive, and promising alternative solution.

Conclusion

The study showed greater in-vivo anthelmintic activity against experimentally infested Sheep using Baber Pole worms (*Haemonchus contortus*) while at the same time did not show any toxic or side effects to the host (Sheep). Therefore, preliminary study suggest that it can be used in practice to control and manage Barber Pole Worm (*Haemonchus contortus*), which will provide sustainable management thereby reducing the use of commercials anthelmintic and slowing down the spread of resistance of Barber Pole worm (*Haemonchus contortus*).

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