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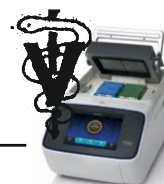

The Effects of Bonny Light Crude Oil (Blco) on the Helminth Parasite, *Heligmosomoides Bakeri (Polygrus)* of Mice.

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ABSTRACT

This study examined the effect of crude oil on Heligmosomoides bakeri in albino mice. A total of 35 albino mice of 5-8 weeks were used for this study. The mice were randomly divided into five groups (A, B, C, D and E) of 5 mice each and infected with 0.13ml of H. bakeri larvae. Mice in the first two groups (A and B) were given crude oil per os at the concentrations of 0.2 and 0.05 mg/ml respectively. Those in group C were given Albendazole and group D infected but not treated. Group E was used as a naïve control group. Two other groups F and G were used for toxicity test. All mice were observed for clinical signs and symptoms of abnormality all through the study and all parameters were assessed following standard procedures for 5 weeks. Administration of crude oil per os to mice in groups A and B at the dose levels used in this study showed no significant ($p>0.05$) changes in PCV, body weight and fecal egg counts of the mice. There was a significant ($p<0.05$) difference in larval mortality test between the crude oil treated groups compared to the control group by their movement (if normal, moving or dead i.e. no observed motion within 10 mins). The Albendazole group C had the least worm burden and fecal egg count compared to the groups treated with different concentration of Crude oil. In conclusion, it may be suggested that heavily infected animals may not respond to treatment with crude oil against nematodes which discredit the unorthodox folklore medicine for parasitic treatment by some rural. Its effect on the worms appeared to be transient.

Key words: Heligmosomoides bakeri, mice, Albendazole, Bonny Light Crude Oil

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INTRODUCTION

One of the major environmental problems for all aquatic organisms is crude oil spillage [1]. This crude oil spillage constitutes a very important threat to both terrestrial and marine fisheries, wide life, invertebrates, birds and mammals and their habitats, [2,3]. Ugbong, [4] reported that crude oil and its products hasten the death of marine fishes in aquatic ecosystem. Following any oil spill, a number of simultaneous processes occur: spreading, dispersion, volatilizations, evaporation, photo-oxidation, emulsification, sedimentation and biodegradation, which together determine the fate of the constituent hydrocarbons [5].

Crude oil and refined oil's major effects on the environment is dependent on some factors either individually or in combination [6]. Its toxic effects are seen on different organs such as kidney, lungs, liver and even the skin [7,8]. For many years, raw crude oil in particular Naftalan crude has been used in the treatment of skin disorders, venereal diseases, internal disorders, sugery and oncological conditions. Some of the oils that are being used while having general therapeutic properties (analgesic, desensitizing, bacteriostatic, bacteriocidal, regenerative), do not have any blastomogenic effects [9]. The treatment of skin disorders using crude oil has been reported to have toxicological effects [10], but some rural individuals consume it and its products orally as unorthodox folklore medicine for parasitic treatment.

Heligmosomoides bakeri (H. bakeri) is an economic important nematode of microtine and murine rodents which causes chronic infections due to its multiple immunomodulatory mechanisms by suppressing the immune response [11]. This nematode is a *Trychostrongylidae* family, where male and female worms can be morphologically distinguished [12]. The life cycle which takes about 13-15 days to complete is direct and no intermediate host [13]. The eggs are passed in feces with a well-developed larva which can be observed moving vigorously within the eggshell. Between 1-3 days of hatching and moulting, L_3 is ensheathed, thus, becoming the infective non-feeding stage [12]. About 7 days later, the male and female worms emerge into the lumen of the duodenum. They attach to the epithelial layer of the duodenum where they feed off the content of the gut. The adult worms mate and eggs are shed in feces. The complete life cycle from egg to egg takes a minimum of 15 days and the female worm will live inside their host for 8 months. These worms form cysts in the wall of the intestine. *H. bakeri* is a temperate parasite and survives under 4-10°C. In tropical regions, they are maintained in refrigerator temperature between 4-10°C. They can hardly survive outside the normal temperature except within the host, that is during infection.

These worms often form cysts in the wall of the intestine. These cysts often become infected with bacteria, but it is not yet known if they are harmful to the host. The terminal end of the infection could result in death, depending on the dose of infection to the animal. Some animals are known to be immuned to the infection after an initial immune suppression which leads to loss of weight, low Packed Cell Volume (PCV), and or increase White Blood Cell (WBC) count. The aim of this study is to determine the degree of worm establishment in animal dosed with different concentrations of crude oil.

MATERIALS AND METHODS

The crude oil used for this study was obtained from the Escravos terminal of Chevron PLC. The crude oil was stored in a clean container and kept in the laboratory until it was required for use. The composition and physiochemical property of the crude oil is presented in the table below.

Table 1: Composition and physical properties of Nigerian crude oil

Property of components	Contents
API gravity (20°C)	33.50
Sulphur (wt. %)	0.14
Nitrogen (wt.%)	0.23
Nickel (mg/kg)	5.40
Vanadium (mg/kg)	1.90
Naptha[a] (wt. %)	21.50
Cycloalkanes	6.70
Alkanes	11.70
Aromatic Hydrocarbons	3.10
High boiling fraction[b] (wt. %)	73.80
Saturates	33.00
N-alkanes	4.60
Isoalkanes	12.50
Aromatic Hydrocarbons	23.70
Polar materials (wt. %)	0.40
Insoluble materials (wt. %)	2.50

[a]fraction boiling from 20-205°C,

[b]fraction boiling above 205°C

Preparation of the oil before use

Its exposure to sunlight in shallow pans for 24hrs is to allow the extremely light and volatile fractions to evaporate such as Aromatic Hydrocarbons, hydrogen sulfide, benzene, sulfur etc., leaving behind the stable components. The product stimulates the naturally occurring condition following spillage [14].

Thirty-five (35) albino mice obtained from the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka were used for the study. The mice were distributed into 5 groups each consisting five (5) mice and housed in galvanized metal cages. The mice were labelled for identification using 1% Picric acid.

Experimental design:

All groups except group E-G was infected with 0.13ml which contains $200L_3$

Group A- Infected with *H. bakeri,* and treated with 0.2mg/ml of BLCO.

Group B- Infected with *H. bakeri* and treated with 0.05mg/ml of BLCO.

Group C- Infected and treated (Standard – Albendazole) (+ve control)

Group D- infected and untreated (-ve control)

Group E- Uninfected control (naive mice)

Group F- Uninfected (0.5 ml low BLCO for acute toxicity)

Group G- Uninfected (1ml high BLCO for acute toxicity)

The mice were housed in clean metal cages, in the fly - proof experimental animal house of the Department of Veterinary Parasitology and Entomology. They were acclimatized for 4 weeks and fed with commercially formulated pelleted layer's mash (Vital feed®). They were given clean water and fed *ad labium* throughout the study. The cages were cleaned every 2

weeks properly. The base line parameters were taken before infection with *Heligmosomoides bakeri*, L_3.

Culturing of *H. bakeri*

Fecal cultures of *H. bakeri* were made from infected donor mice in the department of Parasitology and Entomology. The cultures were made by emulsifying the fecal pellets and filtering, removing the debris. It was washed and sedimented by centrifugation. The sediment obtained was smeared as thin layers on moist filter papers inside Petri-dishes. Ammonia gas from the sediment was allowed to escape for 10 minutes before covering the dishes. The cultures were kept in the refrigerator at 4°C for 7 days and monitored daily against drying up and moistened if dryness was observed.

Larval (L_3) Recovery from culture

Larval recovery was done by gradual pouring of water on the culture with the Petri dishes slanted so that the larvae would swim down. A Pasteur pipette was used to harvest the larvae into a covered tube and later kept in a refrigerator at 4°C for the larvae to settle down.

Larval concentration and larval counts

After about 10-15 minutes, excess water was removed from the stored larvae with the aid of Pasteur pipette. The number of larvae actually administered to each mouse was estimated from direct count of the number present in each of the volume (20µl) expelled onto the surface of a microscope slide of which the average was taken to estimate the number of the infective larvae. The dose of 200L_3 (infective larvae) in 0.13mls was administered using automatic micropipette filled with slightly curved blunt needle according to the method of Fakae, 2001 [15].

Acute Toxicity Test

Ten (10) mice were used for this test. They were those in group F and G. 0.5 ml and 1 ml of crude oil was administered to each mouse in cage F and cage G respectively. They were closely monitored for 3 days. Two died instantly due to injury from the two cages. No death was recorded afterwards and the remaining showed no sign of toxicity.

In vitro Larval Mortality Test

Anthelmintic efficacy of two concentrations (0.5 and 1 mg/ml) of crude oil was also determined by mortality of the *H. baker* L_3. Distilled water was used as the negative control. An average of 16 larvae in 50µl was put in each Petri dish soaked with 0.5ml and 1ml of crude oil in filter paper. Each concentration was replicated three times. After, the plates were stored in the refrigerator at 4°C and examined under light microscope at 6, 12, 18, 24, 30, 36 and 42hrs. Larvae were classified as normal if moving or dead, that is if no observed motion took place during a 10 seconds interval to a source of light.

Mortality was calculated as:

$$\frac{\text{mean number of survived larvae at Y concentration}}{\text{mean number of survived larvae in distilled water}} \times \frac{100}{1}$$

Inoculation of *H. bakeri* L_3 into the mice
200L_3 of *H. bakeri* were administered to each mouse *per - os* with a microtitre pipette

Larvae acclimatization
After harvest of the larvae, they were stored in the refrigerator to acclimatize them between 24-48 hrs before being used to infect the mice.

Parameters determined:

A) Fecal Egg Count:
Following patency of all the infected mice tested by the flotation technique, FEC were conducted on faeces collected daily in the mornings till the end of the experiment by place each mouse in a plastic bowl and allowed to defecate within minutes and mice allowed to return to their cage. Fecal egg counts were conducted using both flotation and modified McMaster techniques [16] for low and high FEC respectively. The mean number of eggs for each day and for each group was determined by simple arithmetic means, the egg per Grammy (EPG) for individual mice were summed and the total divided by the number of mice to give the mean EPG for each day of sampling.

B) Packed Cell Volume (PCV %) Determination
The percentage Packed cell volume (PCV) of all the mice in each group was determined weekly from day zero (D0) till the end of the experiment.

C) The weight of the individual:
The weight of the individual animal in each cage was taken and the average weight of each group was determined using a top loading balance (Sartorius GMBH, Gottingen, Germany). This parameter was taken weekly.

D) *In vivo* Experiment (post mortem):
Post mortem worm counts procedures were according to the methods of [17] Ngongeh (2008). Briefly, each mouse was sacrificed with diethyl-ether and the gastrointestinal tract was quickly removed. Up to three quarters or the entire length of the small intestine starting from the duodenum was opened by cutting along its longitudinal axis with a pair of fine scissors. The adult worms from the intestine were recovered by suspending each intestine on a fine thread and dipping into Hanks balanced salt solution (HBSS) in a universal bottle, and the thread supported by fastening its free ends between the universal bottle and its cap, and then incubated at 37°C. This arrangement allowed the worms to drop freely to the bottom of the bottle after leaving the intestine. Within 2 to 3 hrs all worms would have migrated into the saline. The intestine was then discarded, after little agitation to ensure that no worm was being trapped by any two apposed sections of the gut, and fresh saline added. After removal of the intestine the incubation of the worm suspension continued overnight (20 h) to ensure complete disentanglement of the worms. At the end of the overnight incubation saturated sodium chloride was added to the Hanks saline containing the worms to make up a 30% v/ v solution. The worms then died within 30 min of this treatment, relaxing the tight spiral coils characteristic of the live worms, thus making counting easier. The volume of the suspension was reduced to 5 ml and poured into a clean ruled Petri dish. All the worms present in each sample were counted individually noting their sexes.

Post mortem worm count procedures were conducted according to [18]. The female had 12-15 coils and the male 8-12 coils. The male was distinguished from the female by its' prominent copulatory bursa and two long thin spicules at the posterior end which is not always found in the female.

Statistical analysis: This was carried out using SPSS version 12.0.1 for Windows, as described by Behnke *et al.,* [19]. Where data conformed to

normal distribution, analysis was by Analysis of Variance (ANOVA) in general linear model (GLIM) and results were summarized as arithmetic means with standard errors of means (SEM). Those parameters, which were recorded on more than one occasion namely, body weights, FEC, and PCV were analyzed by repeated measures ANOVA (rmANOVA) in GLIM. Where data did not conform to normal distribution, appropriate logarithmic transformations namely, Log10(X+25) for FEC [19] and Log10 (X+10) for worm counts [15, 20] were adopted prior to analysis and all residuals for ANOVA were checked for approximately normal distribution. These data were summarized as mean log values ± SEM. Correlations between variables were analyzed by Spearman's Rank Order Test. Probabilities of 0.05 or less were considered significant.

Ethical consideration: The ethical conditions governing the use and conduct of experiments with life animals [21], were strictly followed and the experimental protocol was approved by University of Nigeria, Nsukka Animal Ethics Committee.

RESULTS

In vitro larval mortality tests

The result of the larvicidal efficacy of the crude oil showing the means of the survived larvae at two concentrations (0.05ml and 1ml) of crude oil after 6, 12, 18, 24, 30, 36, and 42hrs exposures. Larval mortality was highest, lower and least at 1ml, 0.5ml and distilled water respectively. Between the two crude oil groups, mortality was higher at 1ml. the result showed that there was a significant difference between the two concentrations throughout the test.

Acute Toxicity Test: The result of the acute toxicity test of crude oil which was given to 5 mice each in cages A and B at the dose of 0.5ml and 1ml respectively showed no obvious signs of toxicity which should include oral irritation, salivation and champing of jaws. This is followed by coughing, choking, stomach upset, vomiting leading to aspiration, diarrhea, severe depression, anemia.

Body weight:

The body weights of the mice of all the groups were significantly the same in week 1, except group B which was higher than others. In week 4 of infection, mice in group E gained more weight than others but not significantly different ($p<0.05$) from group C. During treatment, the average weight of mice in group E was significantly higher than others. Towards the end of the experiment, groups A, B, D and E gained weight.

Packed Cell Volume (PCV)

The mean PCV of all the mice used for this study was not significant ($p>0.05$) during pre-infection period. At weeks 3 and 4, the PCV of groups A, B, C and D were all lower than group E (naïve mice) which continued to increase as the experiment progressed. Within the last week of infection, groups A, B, C & D had slight increase in PCV values, though significantly lower than group E.

Fecal egg count (FEC)

Similarly, the fecal egg count in all the groups increased during the pre-treatment period. During the treatment period, group D was significantly higher ($p<0.05$) from group C, but no significant difference when compared to group A. On days 7 and 8 post infection, group D was significantly higher from groups A, B and C with a higher FEC of 14.51 and 12.35 respectively. Towards the tail end of the experiment, group C had the least FEC compared to the other groups. Among the crude oil treated mice, those in group B had the highest FEC, but with no significant difference when compared to group A. This is shown in figure 4 below.

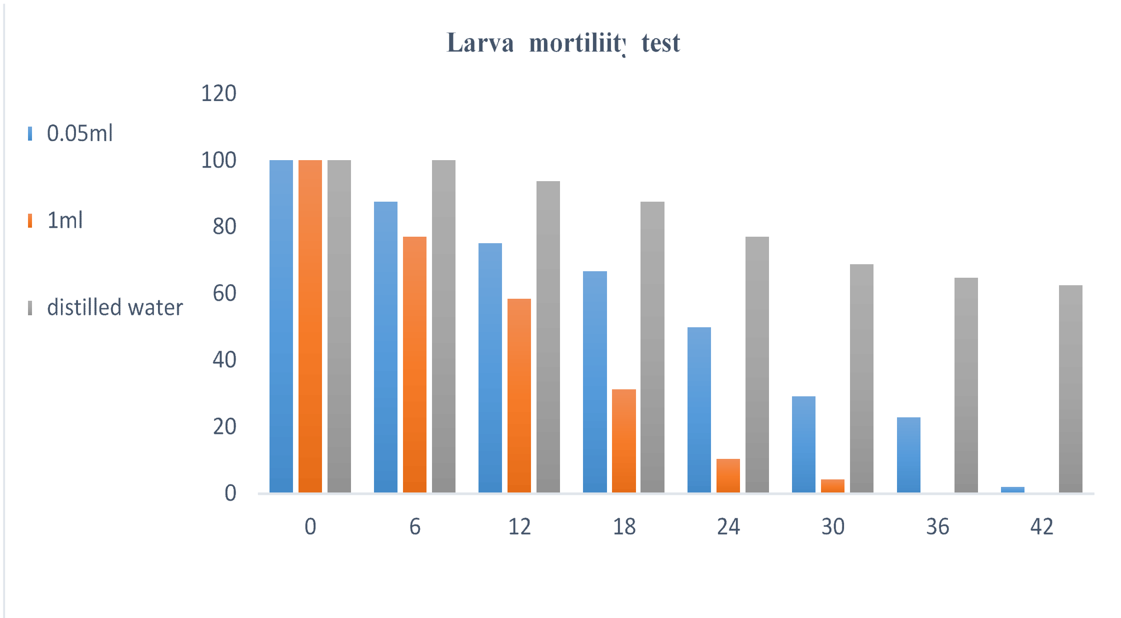

Figure 1. Larval mortality test showing the mean percentage larvae of *H.bakeri* remaining after treatment with different concentrations of Bonny light crude oil

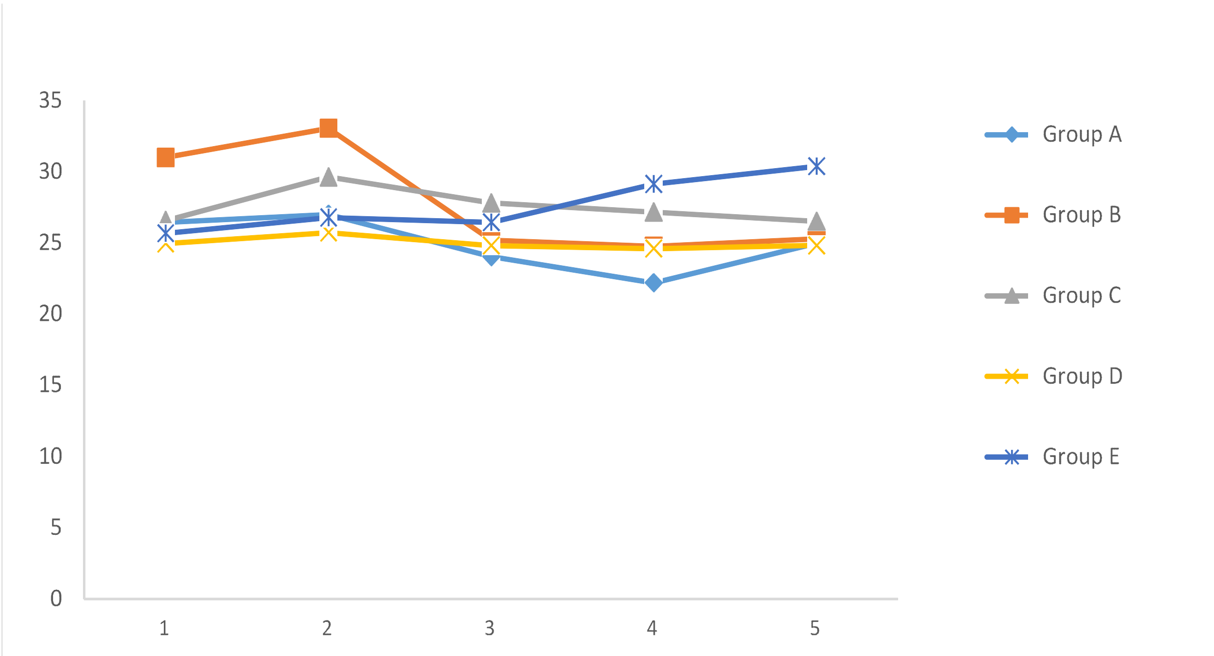

Figure 2. The body weight of mice infected with *H.bakeri* and treated with either Albendazole or different concentrations of Bonny light crude oil.

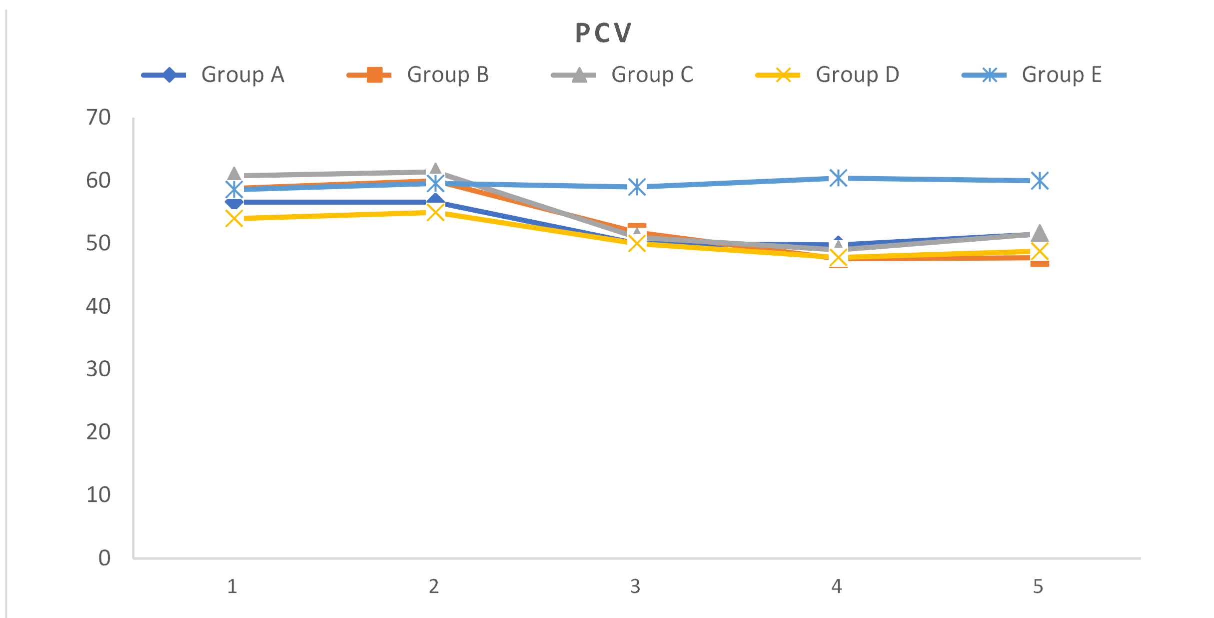

Figure 3. The packed cell volume of mice infected with *H. bakeri* and treated with either Albendazole or different concentrations of Bonny light crude oil.

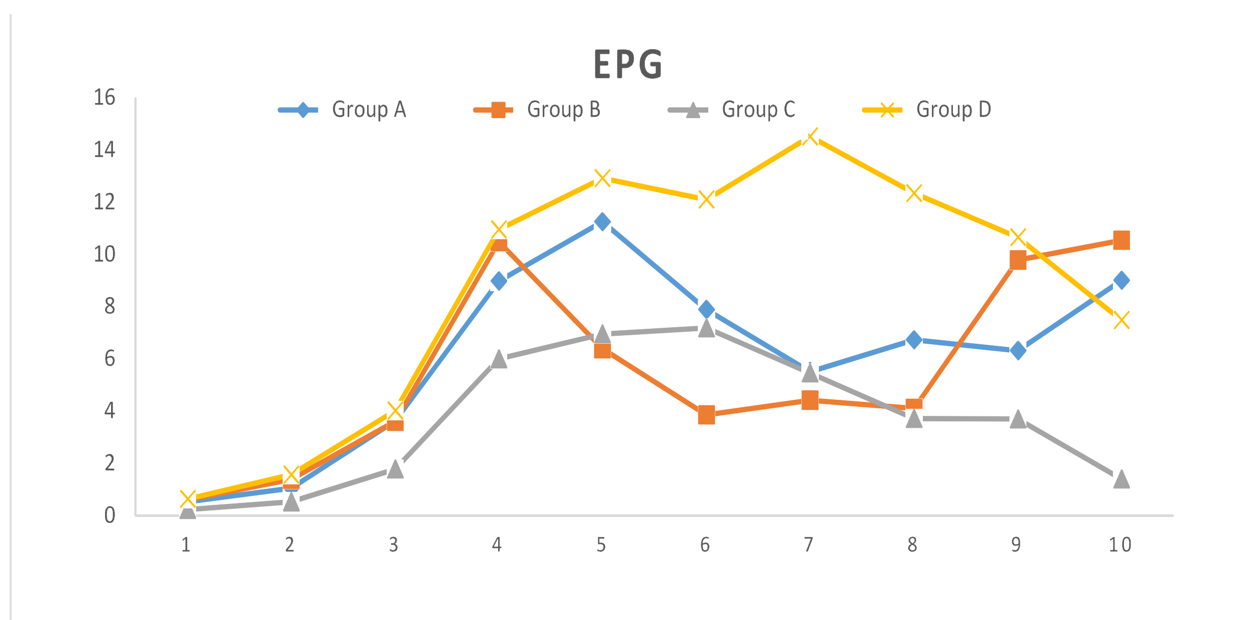

Figure 4. The egg per gram of mice infected with *H. bakeri* and treated with either Albendazole or different concentrations of bonny light crude oil.

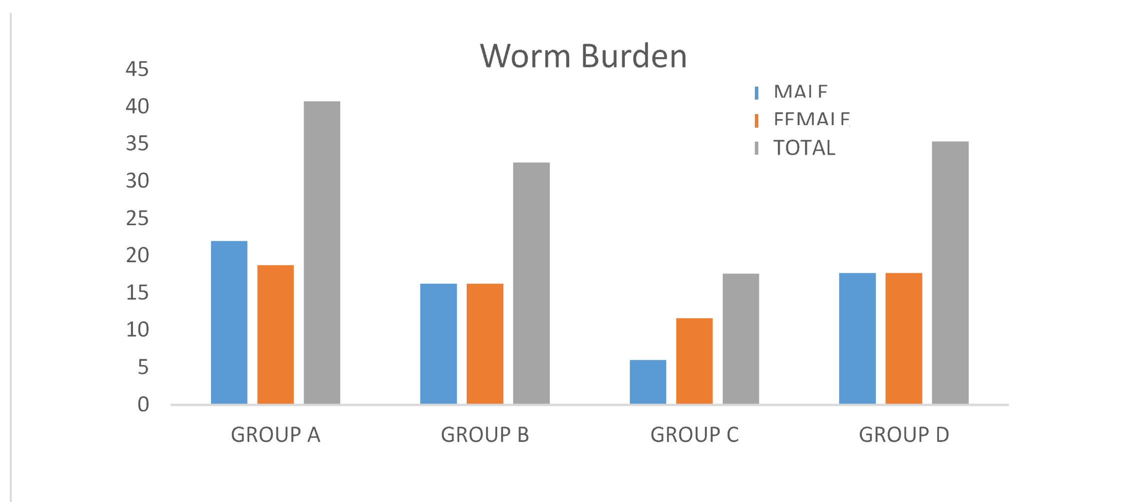

Figure 5: Worm burden of mice infected with *Heligmosomoides bakeri* and treated with either Albendazole or different concentrations of Bonny light crude oil.

Table 2: Comparison between final egg burden and worm burden.

Groups	A	B	C	D
Worm burden	40.75 ± 4.70^{ac}	32.50 ± 6.17^{abc}	17.60 ± 2.93^{b}	35.33 ± 11.57^{bc}
Final egg count	9.01 ± 1.24^{a}	10.54 ± 3.31^{a}	1.40 ± 0.45^{b}	1.40 ± 0.45^{a}

DISCUSSION

The larval mortality test result showed that the two concentrations of crude oil eventually led to the death of the larvae. The larvae treated with lower dose appeared to stay longer before death.

The acute toxicity test showed no side effects on the mice; only a slight change in the feacal consistency (mucoid feaces). This was however not considered to be of significant consequence.

The body weight of all infected mice tended to be lower than those of the control group. Uninfected group E (naïve) was able to maintain and gain weight throughout the experiment. All experimental animals gained weight during the treatment, though there was no significant difference compared to group E. This significant decrease in weight during the experiment may be attributed to the exposure to crude oil. This is consistent with the reports of [22] who stated that crude oil consumption can induce a significant decrease in body and organ weights.

The PCV of the infected mice was lower than that of the control group E. However, PCV generally fluctuated with obvious depression from week 3 post infection in infected groups. This decrease was most prominent in week 4 resulting in anaemia. This is consistent with [23], who recorded low Hb and PCV in rats fed with petroleum products.

The faecal Egg Counts (FEC) rose fairly in all the groups but the slope of regression line in group C (Albendazole) i.e. positive control, depended on administration of treatment, and was steeper in treated groups than in untreated group D. It has been shown that anthelmintic treatment given during a certain period in the course of infection could increase the host's protective immunity to *H. bakeri*. The effect of the treatment was to reduce worm burden and to reduce the faecal egg output. Albendazole was effective primarily against these stages. In group D, egg production may have been depressed by host immunity and the crowding effect on worm population. If animals are

treated, there is a more rapid decrease of egg production but in this case, the result could lead to the utilization of variations of egg production as a criterion for estimating host-parasite relations [17].

An increase in worm burden resulted to a decrease in egg count. This can also be attributed to overcrowding of worms. In comparing the two concentrations of crude oil, group A WB was higher than group B WB, but group B had a higher FEC than that of group A. Group C had the least WB and FEC. This means that Albendazole had the greatest effects on the worms among the treatment groups. The faecal egg count of Group D is lower compared to groups A and B but the WB was higher than that of group B. This showed that the immunity of the animal may have played a role here or that the overcrowding effect of the worms reduced the faecal egg output.

Conclusion

The result of this study showed that individual mice responded differently to the infections of *H. bakeri*. It was also observed that crude oil had a temporal effect on the parasite. This is so because the groups treated with different concentrations of crude oil could have adapted to the effect of oil and subsequently the FEC increased from D8. Therefore, the anthelmintic property of the crude oil was either not beneficial or that the dose probably was not high enough to knock down the parasite completely. Moreover, animals exposed to crude oil experienced some side effects according to [24]. From the fore going therefore, we can say that the use of crude oil to alleviate gastro-intestinal disorders as claimed by many may exclude gastrointestinal nematodes based on the findings of this work.

We however suggest further work to be done at a higher concentration and probably with an extended treatment regimen with crude oil either as prophylactic (i.e. before infection) or as therapeutic (immediately after patency) and the effects observed or monitored.

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