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Comparative Studies of Ethanolic and Aqueous Extracts of Bambusa Vulgaris for Anthelmintic Activities in Infected Rabbits

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Abstract: *Ethanolic and aqueous extracts of Bambusa vulgaris leaf were comparatively screened for anthelmintic activities; ethanolic extract appeared to be more potent in expelling the ova of Ascaris worm and the treated rabbits showed rapid weight gain with a mean value of 0.25kg after treatment as against 0.21g during infection than aqueous extracts. These suggest that they could serve as an alternative source of anthelmintic agent to patented anthelminthic drugs.*

I. Introduction

The economic and social impacts of helminthes in sub-Saharan African cannot be underestimated with the effect ranging from stunted growth, reduced weight gain, diarrhea, respiratory problems, reduced productivity and death in livestock especially in the tropics and developing countries (Charlier et al., 2009). This undermines the economic status of those that derived livelihoods from livestock and livestock based industries. Problem of resistance to common anthelminthic agents, availability and prohibitive cost deficient to livestock extension services and the high cost of conventional anthelminhtics, has led to the evaluation of medicinal plants as an alternative source of anthclminthic for treatment of livestock against helminths (Charlier et al., 2009).

In humans, helminths cause innumerable suffering such as gastroenteritis, anemia, stunted growth, blindness and permanent disability like lameness worldwide. Consequently, control of helminthiasis has received major focus in biomedical research and veterinary extension where synthetic drugs have been developed and used (Sebuguzi, 2000). Conversely, many parasites have developed resistance to these drugs while others have had serious side effects in addition to being costly for the poor countries, thus necessitating the search for alternative sustainable control strategies. This has led to increasing demand for herbal medicines worldwide in recent past.

In Nigeria, the economic losses due to helminthiasis in small ruminants alone have been estimated to be at least 144 million naira annually, through death, weight loss, loss of market values and other essential parts (Okun et al., 1980). In addition more than 800 million people in the world are affected by helminthiasis. Helminths are more in tropical regions due to climatic and socio-cultural factors. The major control measure against helminthiasis in Nigeria is chemotherapy; however, the availability of drugs varies (Okun et al., 1980).

The significance of helminthiasis has been recognized by local people and herdsmen from the earliest times who have made various attempts to control them through the use of medicinal plants. Fulani herdsmen in Nigeria recognize animal helminthiasis to be a problem of greatest significance in calves of less than a year old. Only few of the plants used traditionally as anthelminthics in Africa have previously been studied. Hunteria umbellate, which has been used as an anthelmintic for humans in Nigeria, was shown to be potent against Ascaris just as the pure piperazine base (Onuguluchi, 1964).

In addition to the helminth infections which cause direct economic losses due to reduce animal production yet another dimension is added by the fact that several helminth infections can be transmitted to man (zoonoses) with regard to their host range, some helminth species do not discriminate between humans and animals at all while others have complex life cycles which require man as either final or intermediate host in order to complete their lifecycle. Some of these diseases maybe very harmful to man and a considerable amount of effort have over the years been put into the control through meat inspection legislation (Getz, 2011)

The prevalence of helminth diseases in Nigerian is very high especially during the wet season when infection is high as 100% in cattle. Such high infection rates prevent them from attaining optimum productivity especially under the animal husbandry system (Fakae, 1999). Financial costs of internal parasitism are enormous due to increase in mortality and a reduction of growth rate and wool production (Meleod, 2000). Due to increasing cost of treatment particularly in developing economies, and the socio-cultural and environmental factors made the people vulnerable, they tend to rely on plants as substitute for orthodox medicine. Also many diseases are becoming resistant to many therapeutic drugs hence to explore these abundant plant resources to reduce the effect of parasitic worm infections and the need for this study.

Medicinal plant are defined by Mann et al (1993) as any plant in which one or more of its organs or components contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. The use of medicinal plant in traditional medicine is a common and acceptable

practice in Nigeria and many other countries. It is estimated that 66 – 85% of the world population depends directly on plants as medicine (Terry, 2000).

Medicinal plants are of great importance to the medical world. All civilization has always had traditions of using plants to promote healing. Plants remain the basis for the development of modern drugs. It is a common knowledge that even in America today. About 25% of all drug prescription is derived from plants (Wang, 1995). There is a long list of medicinal plants that have been used in the control of helminth worms in different parts of Africa. Such plants include: Combretum mucronatum and Mitragyna stipulosa, (guinea worm); raw garlic (Allium satrum) used in Nigeria as a vermifuge (Ascaridia galli) in chicken, Combretum aromaticum and Combretum medica (Ascaris lumbricoides) (Kaleysa, 1975); V. amygdalina (humans and livestock gastro-intestinal parasites) (Nalule et al., 2011).

II. Materials And Method

The materials used are: Bambusa vulgaris (Bamboo leaves), rabbits, Ketrax-patented drug, distilled water and Ethanol

Plant: The plant Bambusa vulgaris leaves were collected from a bush in Doko village, in Lavun LGA of Niger state of Nigeria. The leaves were then air dried at room temperature in the biochemistry laboratory for four (4) weeks.

Morphological description: Bambusa vulgaris commonly called- Bamboo is one of the fastest growing plants in the World. Bamboo are of notable economic and cultural significance in south Asia, south East Asia and east Asia, being used for building materials, as a food source and as a versatile raw product. Geographically, Bamboo species are found in diverse climates from, cold mountains to hot tropical regions. They occur across east Asia from 50^0N latitude in Sakhalin through to northern Australia and west to India and the Himalayas (Saha et al., 2001). Bambusa vulgaris occurs spontaneously or naturalized mostly on river banks, road sides, waste lands and open ground, generally at low altitudes. In cultivation, it thrives best under humid conditions up to 1000m altitude but tolerates unfavorable condition as well, dry season and also tolerates a wide range of soil types (Loupper et al., 2006).

Bamboo leaves is used for construction of houses, huts, fences, props and furniture; as raw materials for paper pulp, shoots are rarely used as a vegetable to livestock fodder. They are also planted as ornamental or boundary maker, used to support banana plants; split stem used for brooms, baskets in New Guinea, culms are use to make combs and penis gourds and musical instrument (Loupper et al., 2006). The medicinal indications of Bamboo includes: abortifacient- for kidney troubles, treatment of fever and hematuria; treatment of infantile epilepsy (Christopher et al., 2006).

Ketrax- The Ketrax used as control in this study was bought directly from a notable Pharmaceutical store in Bida, Niger state. Ketrax is a versatile anthelminthic agent commonly used as levamizole against gastro-intestinal helminth parasites.

Helminth- The rabbits used for this study were already infected from the farm where they were purchased and confirmed through stool microscopy and the eosinophil count.

Ethanolic extraction of the plant: Exactly100g of the Bamboo leaves' powder were weighed using the digital balance into reagent bottle, 600mls of ethanol was measured using the measuring cylinder and added to the reagent bottle containing the powdered leaves part. It was then stirred vigorously and allowed to stand on the bench for 72 hours for effective percolation and subsequent extraction. The mixture was then filtered using Whatmann No.1 filter paper and the filtrates obtained were evaporated to dryness using water bath.

Aqueous extract: Exactly100g of the Bamboo leaves' powder were weighed using the digital balance into reagent bottle. 600ml of distilled water was measured using the measuring cylinder and added to the reagent bottle containing the powdered leaves part. It was then stirred vigorously and allowed to stand on the bench for 72 hours for effective percolation and subsequent extraction. The mixture was then filtered using Whatmann No.1 filter paper and the filtrates obtained were evaporated to dryness using water bath.

Stool microscopy: In order to determine the health status of the rabbits, stool microscopy of their stool were carried out as described by Chessbrough (2004).

Grouping of the rabbits: The rabbits were grouped into four named: A; B; C; and D. Each of the group had 3 animals, group A were treated with Ethanolic extract, group B were treated with Aqueous extract, group C was the positive control treated with the patented drug-Ketrax while group D was the negative control that were treated with distilled water. The extract was dissolved in distilled water using weight/volume and it was administered with a syringe.

Rabbit weight study: The weights of the rabbits were monitored daily before infection, during infection and after treatment. The rabbit weights were carried out before feeding them for accurate determination of the weight.

Eosinophil count: Following stool microscopy, the eosinophil count was carried out to confirm the result. There is a correlation between increased eosinophil count and parasitic infection (Eosinophilia-Wikipedia, 2014).

III. Results

Table 1: Eosinophils count of animals of the rabbits before and after infection

	Eosinophits count after infection	Eosinophilis count after treatment
A_1	D	D
A_2	0.6×10^9	0.3×10^9
A_3	0.55×10^9	0.3×10^9
B_1	0.4×10^9	0.4×10^9
B_2	0.4×10^9	0.4×10^9
B_3	0.42×10^9	0.38×10^9
C_1	D	D
C_2	0.4×10^9	0.2×10^9
C_3	0.45×10^9	0.28×10^9
D_1	0.3×10^9	0.3×10^9
D_2	0.3×10^9	0.28×10^9

***D = rabbits that died during the study.**

Table 2: Shows the mean weight of the rabbits

Weeks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A_1	0.3	0.35	0.4	D	D	D	D	D	D	D	D	D	D	D	D	D
A_2	0.2	0.3	0.45	0.4	0.35	0.35	O.3	0.25	0.3	0.25	0.25	0.2	0.2	0.25	0.25	0.35
A_3	0.35	0.45	0.55	0.6	0.55	0.5	0.5	0.4	0.35	0.35	0.3	0.3	0.4	0.4	0.45	0.4
B_1	0.45	0.55	0.6	0.65	0.6	0.6	0.55	0.5	0.5	0.45	0.45	0.4	0.4	0.4	0.35	0.3
B_2	0.5	0.45	0.5	0.5	0.5	0.45	0.45	0.4	0.4	0.4	0.35	0.3	0.4	0.4	0.35	0.35
B_3	0.55	0.6	0.6	0.6	0.55	0.55	0.5	0.5	0.45	0.45	0.4	0.4	0.35	0.35	0.3	0.3
C_1	0.6	0.7	D	D	D	D	D	D	D	D	D	D	D	D	D	D
C_2	0.3	0.3	0.4	0.45	0.4	0.4	0.35	0.35	0.4	0.4	0.45	0.45	0.5	0.5	0.55	0.55
C_3	0.45	0.55	0.5	0.55	0.5	0.5	0.45	0.4	0.4	0.45	0.45	0.45	0.55	0.5	0.5	0.6
D_1	0.75	0.75	0.75	0.8	0.7	0.7	0.75	0.8	0.85	0.85	0.9	1.0	1.1	1.05	1.1	1.15
D_2	0.5	0.55	0.55	0.65	0.6	0.6	0.65	0.7	0.7	0.7	0.75	0.8	0.8	0.8	0.85	0.85

KEY
D-Death
Week1-4 indicating period of acclimatization of experimental animal
Week 5-8 showing the period of infection
Week9-12 indicating period of treatment
Week 13-16 showing the period after treatment

IV. Discussion

This study has further confirmed the negative impacts of helminth worms on the growth of ruminant animals. Some of the experimental animals showed an arrested weight, then a gradual decline in weights due to helminth infection. Earlier works had reported that ascariasis and other intestinal helminthiasis often cause stunted and arrested growth through malnutrition (Barnabas et al., 2011; De Silva et al., 2003). The observed weight differences during pre infection, during infection and after treatment with the plant extracts of Bambusa vulgaris were statistically significant ($P<0.05$).

Treatment of the infected rabbits in group A with ethanolic extracts of Bambusa vulgaris showed a rapid recovery in weight after treatment with the mean value of 0.25g as against 0.21g during infection and there was reduction in the eosinophil count (Table 1).Ethanolic extracts had more positive effect on the weight of the experimental animals compared to the aqueous extract. According to Al-Shailbani et al (2008) ethanolic extract of Adhatoda vasica is slightly more effective compared to aqueous extract on eggs and larva of gastrointestinal nematodes.

Treatment of group B with aqueous extract of Bamboo vulgaris also showed slow recovery when compared to the ethanolic extract. The observed difference in the activities of the two extract types might be because ethanol enables the complete extraction of the active ingredients of the plant. Experimental animals in group B showed a mean value of 0.466g during infection period between 5-8 weeks and a mean value of 0.316g after treatment between 13-16 weeks, indicating that aqueous extract of Bamboo leaves may be potent anthelminthic but at reduced rate.

Experimental animals in Group C treated with the patented drug ketrax as positive control also showed speedy weight recovery against the parasite and a faster reduction in the eosinophil count. The use of ketrax is therefore justified as a reliable antihelminthic agent. While experimental animals in group D has clearly demonstrated the negative impacts of intestinal helminth parasites. Although the mechanism or pathological

routes of this parasite was not part of this present study, they might have altered the nutritional balance of their host hence, the observed drastic weight loss and increased eosinophilia.

V. Conclusion

The study has justified earlier claim that Bamboo vulgaris has antihelminthic property. Ethanolic extracts has proved to be more active in ameliorating the impacts of gastro intestinal helminth infections among ruminants. Therefore, with more studies on the mammalian tolerance and toxicity on the plant, they could serve as alternative source to the costly available drugs in the market today.

VI. Recommendations

From the present study the following recommendations are therefore suggested:

- Further studies into Bamboo vulgaris as medicinal plants should be carried out to elucidate more facts about their anthelminthic activities;
- Pytochemical screening and study of Bamboo vulgaris as anthelminthic agent and
- Further study on Bamboo vulgaris should be carried out on to determine their toxicity and possible side effects.

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