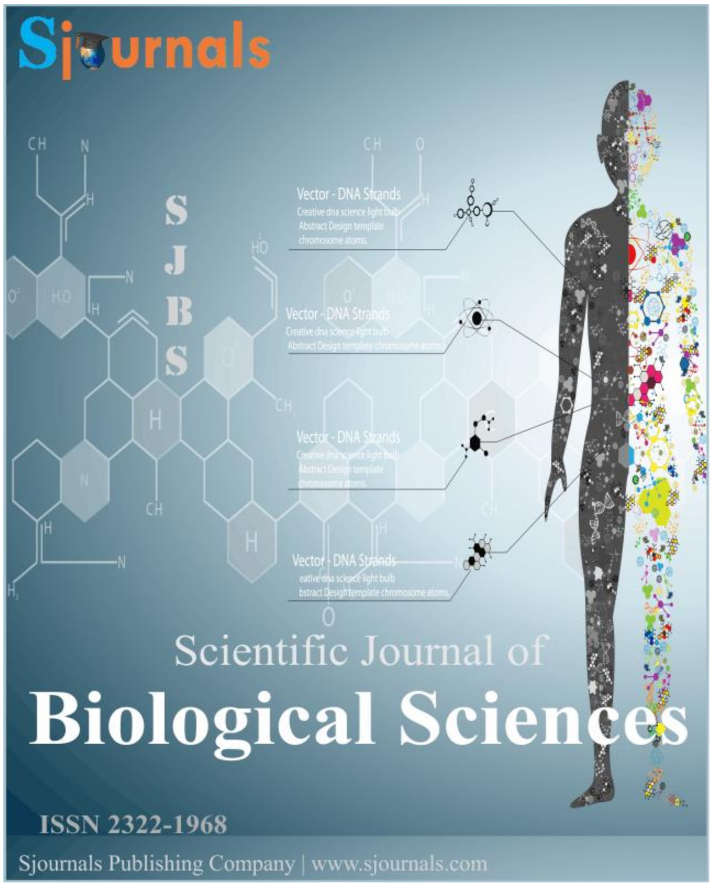
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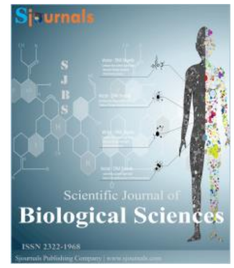

Original article

In vivo anthelmintic effects of *Pterocarpus erinaceus* and *Parkia biglobosa* on *Haemonchus contortus*

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ABSTRACT

The emergence and spread of resistance to chemical anthelmintics in gastrointestinal nematode populations has led to the search for alternative solutions such as the use of medicinal plants with anthelmintic properties. This study was undertaken to examine the in vivo effect of *Parkia biglobosa* and *Pterocarpus erinaceus* on the viability and fertility of adult *Haemonchus contortus* worms in sheep. Fifteen Djallonke sheep, 4 to 5 months old, were divided into three experimental lots. The control lot received no treatment. Two lots were treated with 3.2 g/kg PV of plant powder. *P. biglobosa* significantly reduced egg excretion compared to the control ($p < 0.05$). Both plants significantly ($p < 0.05$) reduced the number of adult worms and the number of eggs per female worm. Numbers of adult worms and eggs per female worm were 330.00 ± 112.84 and 332.0 ± 191.9 in the control lot, 17.50 ± 10.41 and 176.0 ± 96.1 in the lot treated with *P. biglobosa* and 86.25 ± 53.13 and

146.8 ± 90.6 in the lot treated with *P. erinaceus*, respectively. This study showed that *P. biglobosa* and *P. erinaceus* exerted anthelmintic activity in vivo on adult *H. contortus* worms. Further investigations are needed to evaluate the toxicity of these plants and to understand their metabolism in the digestive tract of animals.

1. Introduction

Parasitic nematodes of the gastrointestinal tract represent one of the main constraints related to ruminants rearing on pasture because of the pathological consequences and production losses they cause (Veneziano et al., 2007; Roeber et al., 2013). Epidemiological investigations have shown that haemonchosis is an important parasitic disease in small ruminants in Benin and in particular in the south of the country which is the most watered area (Attindehou et al., 2012). The control of these parasitoses has usually been based on the use of chemical anthelmintics. However, this almost exclusive use of these synthetic molecules faces several limitations nowadays. In developing countries, access to chemical anthelmintics by smallholders is usually limited for financial or practical reasons (Krecek and Waller, 2006). The rapid development of resistance to chemical anthelmintics in worm populations and the global spread of this resistance (Kaplan, 2004; Van den Brom et al., 2015) are the main reasons for the strong impetus to search for alternative solutions (Dèdéhou et al., 2014; Aguerre, 2019).

The evaluation of traditional anthelmintic remedies appears to be a promising field of research. In addition to the use of synthetic molecules, the control of gastrointestinal parasitosis could also be based on the use of bioactive plants, rich in secondary metabolites, and identified as a complementary management solution (Azando et al., 2011; Hoste et al., 2022).

Parkia biglobosa and *Pterocarpus erinaceus*, trees present in several African countries, are exploited for the provision of several outputs: building materials, wood, food, fodder. These plants are also used in the manufacture of many products used in traditional medicine (Zerbo et al., 2011; Tchacondo et al., 2011; Odounharo et al., 2021). Indeed, their barks, leaves, roots, fruits and seeds are used to treat wounds, pain, burns, digestive diseases (diarrhea, dysentery, abdominal pain), cardiovascular, infectious (malaria, abscesses, yellow fever, measles) (Tchacondo et al., 2011; Zerbo et al., 2011; Padakale et al., 2018), etc. Their medicinal properties are believed to be due to the various phytochemical components they contain (Mann and Ogbadoyi, 2012; Abioye et al., 2013).

The present study was undertaken to examine the in vivo effect of *Parkia biglobosa* and *Pterocarpus erinaceus* on the viability and fertility of *Haemonchus contortus* adult worms in sheep.

2. Materials and methods

2.1. Study environment

The phytochemical screening of the powders of the two plants was carried out at the Laboratory of Pharmacognosy and Essential Oils of the Institute of Applied Biomedical Sciences (ISBA) in Cotonou, Benin. The animal test was carried out on the farm of the Faculty of Agronomic Sciences of the University of Abomey-Calavi, located in the south of Benin while the parasitological tests were carried out at the Laboratory of Ethnopharmacology and Animal Health of the University of Abomey-Calavi in Benin.

2.2. Collection of plant material

Plant material consisted of *P. erinaceus* leaves and *P. biglobosa* fruit pods harvested when mature in the Collines department located in central Benin. It was authenticated at the National Herbarium of the University of Abomey-Calavi under the numbers AA6368/HNB and AA6385/HNB respectively. After drying at room temperature for two weeks, they were reduced to powder.

2.3. Phytochemical screening

Qualitative tests were performed for the detection of active chemical compounds in both plants powders. The tube reaction method based on staining or precipitation reactions performed on the fresh or dry powdered plant drug was used. Depending on the family of the compound to be investigated, different reagents were used according to the protocol described by Houghton and Raman (1998).

2.4. Animal management

Animal material consisted of fifteen Djallonké sheep aged 4 to 5 months at the beginning of the experiment. They were identified with numbered wooden tags, housed in well-ventilated pens and fed dry *Panicum maximum* fodder, cottonseed or palm kernel cake. They were given water and lickstone ad libitum. Prior to the experiment, all animals were dewormed with albendazole at a dose of 5 mg/kg live weight and received a deworming bath. They were divided into 3 homogeneous experimental batches of 5 animals placed in absolute confinement in separate pens. An initial quantitative coproscopy ensured that the animals were free of gastrointestinal nematodes and biweekly quantitative coproscopies monitored the evolution of fecal excretion after infestation.

2.5. Experimental protocol

- On day zero (D0), the animals were infested with 2000 infesting larvae (L_3s) of *H. contortus*.
- 1st treatment: 26 days after infestation, two batches of animals were treated with the plant powder administered orally with a little water in one dose, for 3 consecutive days, i.e. on D26, D27, D28, as follows:

lot 1 = positive control lot without treatment (control lot),
lot 2 = *P. biglobosa* lot treated with 3.2 g/kg of *P. biglobosa* powder
lot 3 = *P. erinaceus* lot treated with 3.2 g/kg of *P. erinaceus* powder,

- 2nd treatment: The previous treatment was repeated two weeks after the 1st one, namely at D42, D43, D44, according to the same modalities as described above.
- Sacrifice: one week after the second treatment, i.e. at D51. 4 animals were sacrificed in each batch.

2.6. Variables and statistical analyses

Two types of measurements were taken: indirect measurements based on fecal egg excretion and hematocrit and direct measurements based on parasite counts. The number of eggs per gram of feces (EPG), hematocrit, number of adult worms in the abomasum and the number of eggs per female worm were the parameters measured.

Coprological examinations were biweekly and Eggs per gram (EPG) was determined following the Mc Master technique (Hansen and Perry, 1995). Hematocrit was measured once a week. Animals were sacrificed one week after the second treatment at D51. The number of adult worms was counted in the abomasum. Individual fertility of female worms was determined by the technique described by Kloosterman et al. (2006). Comparison between treatments was performed by a one-criteria analysis of variance with Minitab software.

3. Results

3.1. Phytochemical screening

Chemical screening of *P. erinaceus* leaf powder and *P. biglobosa* fruit pods revealed that they contain many bioactive compounds (Table 1).

The fruit pods of *P. biglobosa* contain main families of compounds such as phenolic derivatives (tannins, flavonoids, flavones, leuco-anthocyanins), saponosides, mucilages, reducing compounds, steroids. The leaves of *P. erinaceus* contain tannins, mucilages, saponosides and cardenolides. Flavonols, flavonones, anthocyanins, alkaloids, quinone derivatives, coumarins, free anthracenics, compound anthracenics, and cyanogenic derivatives are not present in *P. erinaceus* leaves or in *P. biglobosa* fruit pods. Note also that steroids are present in *P. biglobosa* fruit pods and absent in *P. erinaceus* leaves, while cardenolides are present in *P. biglobosa* fruit pods and absent in *P. erinaceus* leaves. Catechic tannins, flavonoids and Flavones are present in *P. biglobosa* fruit pods, and absent in *P. erinaceus* leaves

Table 1
Phytochemical analysis of *P. erinaceus* leaves and *P. biglobosa* fruit pods.

Chemical compounds	*Parkia biglobosa*	*Pterocarpus erinaceus*
Tannins	+	+
Catechic tannins	+	-
Gallic tannins	+	+
Flavonoids	+	-
Flavones	+	-
Flavonols	-	-
Flavonones	-	-
Anthocyanins	-	-
Leuco-anthocyanins	+	-
Alkaloids	-	-
Quinone derivatives	-	-
Coumarins	-	-
Mucilages	+	+
Reducing compounds	+	-
Saponosides	+	+
Free anthracenics	-	-
Compound anthracenics	-	-
Cyanogenic derivatives	-	-
Steroids	+	-
Cardenolides	-	+
Triterpenoids	+	+

- : Absence; + : Presence.

3.2. Effect on egg excretion

In the different batches, egg excretion levels were similar before treatment, i.e. at D24. *P. biglobosa* powder decreased the egg excretion level after each treatment. The difference from the control was significant ($p < 0.05$) after the second treatment. With *P. erinaceus*, the reduction in egg excretion was not significant ($p > 0.05$). In this batch, fecal egg excretion decreased after the first treatment but was not sensitive to the second treatment after which it gradually increased. The rate of reduction in fecal excretion increased from 63% to 93% for *P. biglobosa* and from 17% to 59% for *P. erinaceus* between D29 and D50, respectively (Figure 1).

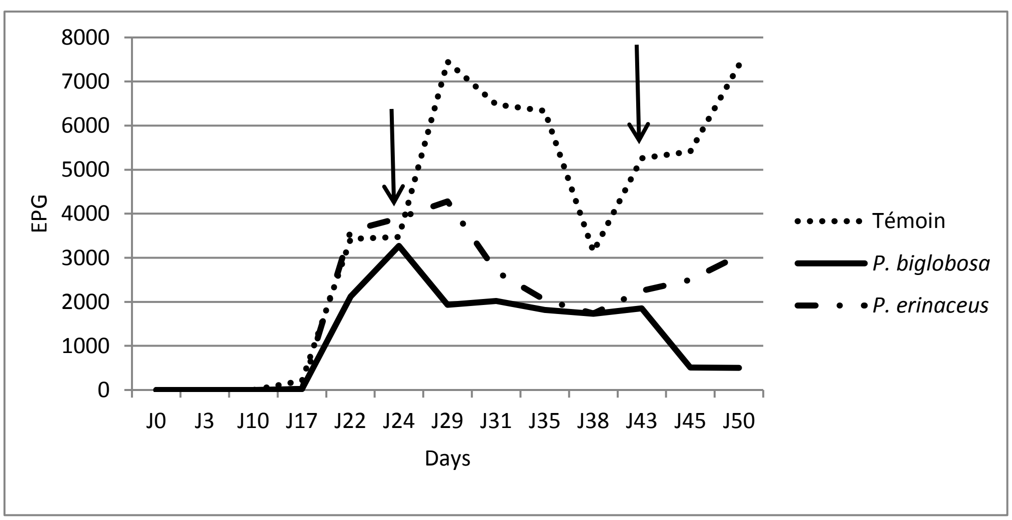

Fig. 1. Variation in faecal egg counts in sheep before and after treatment.

3.3. Effect on hematocrit

Before treatments, slight fluctuations in hematocrit were noted in all batches. After the first treatment, hematocrit initially decreased in treated batches and significantly more in sheep treated with *P. erinaceus*. In a second phase, i.e. shortly before and after second treatment, it increased. During this period, hematocrit was constantly decreasing in control lot. Hematocrit of treated sheep was generally higher than that of control animals with a significant difference ($p < 0.05$) after second treatment at D45 (Figure 2).

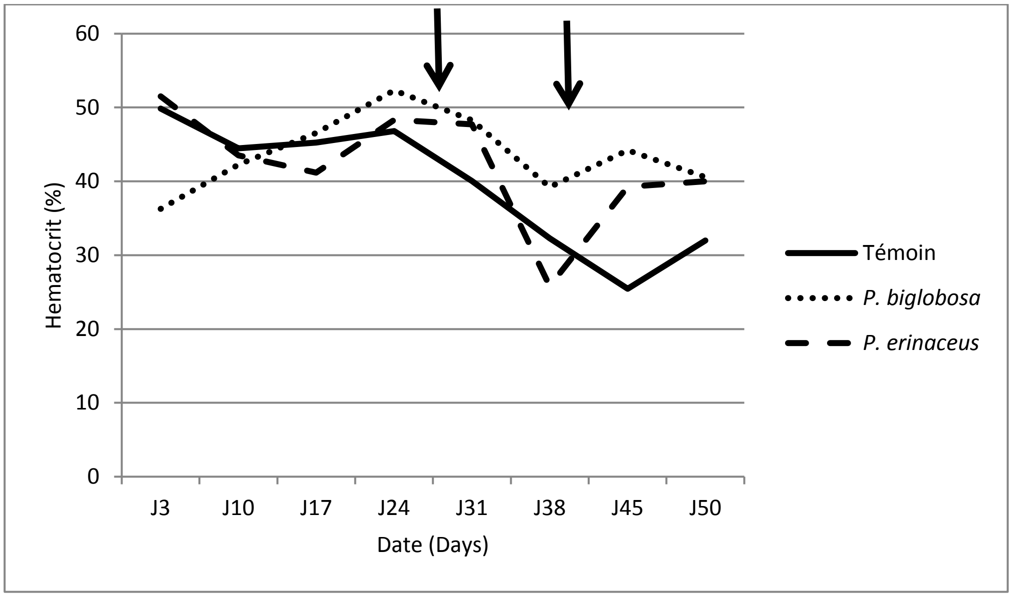

Fig. 2. Mean of haematocrit values in groups of sheep before and after plants treatment.

3.4. Effect on viability of adult worms

P. biglobosa and *P. erinaceus* powders were effective on viability of *H. contortus* adult worms by significantly reducing their number at post mortem examination in sheep (Table 2). Thus, 17.50 ± 10.41 and 86.25 ± 53.13 are numbers of adult worms found in *P. biglobosa* and *P. erinaceus* batches, respectively, compared to 330.00 ± 112.84 for control batch.

Table 2
Variation of the number of adult worms in sheep after treatment.

Treatments	Concentration (g/kg Live weight)	Number of female worms	Number of male worms	Total of worms	Effect on viability
Control	00	147.50^{a} ± 67.02	182.50^{a} ± 65.00	330.00^{a} ± 112.84	a
Parkia biglobosa	3,2	16.00^{b} ± 11.28	1.50^{b} ± 3.00	17.50^{b} ± 10.41	b
Pterocarpus erinaceus	3,2	68.75^{ab} ± 46.97	17.50^{b} ± 10.41	86.25^{b} ± 53.13	b

Values in the same column followed by different letters are significantly different at $p < 0.05$.

3.5. Effect on fertility of adult female worms

In sheep and at the tested dose, powders of both *P. biglobosa* and *P. erinaceus* significantly reduced the number of eggs per female worm of *H. contortus* proving an activity on their fertility (Table 3). The numbers of eggs per female worm were 176.0 ± 96.1, 146.8 ± 90.6 and 332.0 ± 191.9 for *P. biglobosa* and *P. erinaceus* and control, respectively.

Table 3
Variation of the number of eggs per worm female after treatment.

Treatments	Concentration (g/kg Live weight)	Eggs per female worms	Effects on fertility
Control	00	332,0^{a} ± 191,9	a
Parkia biglobosa	3,2	176,0^{b} ± 96,1	b
Pterocarpus erinaceus	3,2	146,8^{b} ± 90,6	b

Values on the same column followed by different letters are significantly different at $p < 0.05$.

4. Discussion

Phytochemical analysis indicates that *P. biglobosa* fruit pods contain major families of compounds such as phenolic derivatives (tannins, flavonoids, flavones, leuco-anthocyan), saponosides, mucilages, reducing compounds, steroids. *P. erinaceus* leaves contain tannins, mucilages, saponosides and cardenolides. These results are similar to those obtained by Bukar et al. (2010) who reported the presence of tannins, flavonoids, reducing sugars and flavones in aqueous and ethanolic extracts of *P. biglobosa* fruit pods. Ouédraogo et al. (2011) also reported the presence of tannins and saponosides in *P. erinaceus*. However, flavonoids, triterpenes and steroids were not found in this study. The differences in chemical composition could be explained by differences in soil conditions, particularly in harvesting area.

The secondary metabolites of the plants would be responsible for their therapeutic properties (Azando et al., 2011; Dèdéhou et al., 2018). *P. biglobosa* fruit pod powder reduced fecal excretion of *H. contortus* eggs and both plants powders reduced the number of adult worms and the number of eggs per female worm. Reduction in EPG levels reflects the reduction in the number of adult worms or the disruption of female worm fecundity. The results obtained with *P. biglobosa* and *P. erinaceus* on worm viability confirm those previously reported in vitro by Dèdéhou et al. (2014) on *H. contortus* adult worms. The decrease in female worm fertility noted in this study has previously been reported with other tropical plants with anthelmintic properties. This is the case with *Z. zanthoxyloides* (Azando et al., 2011) and *Chenopodium ambrosioides* (Maiga et al., 2020). According to Pfeiffer (2007), the size of the uterus of female worms, thus their fecundity is to be correlated with their length. A short length means that oviposition is low.

Several molecules of the chemical families present in *P. biglobosa* and *P. erinaceus* have been suggested in previous works as having antihelminthic properties. These molecules, alone or in combination, could explain this drop in infestation. These include triterpenes and alkaloids in sheep (Githiori et al., 2006; Chagas et al., 2008), saponins (Deepak et al., 2002) and condensed tannins in sheep and goats (Terrill et al., 2007; Dèdéhou et al., 2018). Many previous results suggest that the use of plants rich in condensed tannins or agro-industrial by-products containing tannins in general is the most promising in the field of plants or substances of natural origin with antihelminthic properties (Hoste and Chartier, 2002; Hoste et al., 2022) but the potential role of other secondary metabolites cannot be ruled out (Chagas et al., 2008; Dèdéhou et al., 2018).

The hematocrit values recorded at the beginning of our study are higher than those reported by other authors (Hounzangbé-Adoté, 2004; Chaudary et al., 2007). This variation in hematocrit values could be related to feeding. Attindehou et al. (2012) found that there was no proportional impact of parasite load on the degree of anemia across seasons. In dry season, moderate infestations significantly increased the anemia rate, while in wet seasons, intensity of infestation did not seem to have much influence on the degree of anemia. Wet months are characterized by the availability of forage and the animals can compensate for spoliation caused by worms. Hematocrit values dropped after the 24th day post-infestation. According to Khan et al. (1988), a drop in hemoglobin concentration is observed three weeks after infestation which is equivalent to the beginning of the patent period. Treatment with both plants generally induced a superiority of hematocrit values of treated animals over controls. Increase in hematocrit values could be due to the decrease in worm population in animal's abomasum, in accordance with the results reported by Alam et al. (2020). These authors found that sheep naturally infected with *H. contortus* (mild, moderate and heavy infection) showed a significant decrease in the number of red blood cells, haemoglobin concentration, packed cell volume compared to healthy animals control animals.

Increase in hematocrit could be due to the fact that plants would possess anti-anemic properties that would prevent anemia related or not to parasitism (Mohammed et al., 2021). These plants could therefore have an effect on the resilience of animals to parasitism.

As with any active substance, the effectiveness of plants depends on the concentrations used. For the control of gastrointestinal parasitosis by plant powders or extracts, several concentrations have been used by different authors (Azando et al., 2011; Akouèdégni, 2013; Khan et al., 2018). Concentration of 3.2 g/kg live weight of powder used in this study was effective on *H. contortus*. This same concentration used by Azando et al. (2011) was effective on *H. contortus* but its effect was not significant on *T. colubriformis* and *O. columbianum*. This could be related to the differences in concentrations of bioactive substances in the digestate in the different portions of the digestive tract. Molan et al. (2000) estimated daily amount of condensed tannin in the maintenance ration of forage sheep to be approximately 30-120 g through the digestive tract.

5. Conclusion

P. biglobosa fruit pods and *P. erinaceus* leaves are two tropical plants containing several families of bioactive compounds. They showed in vivo anthelmintic activity on *H. contortus*, a widespread, hematophagous and highly pathogenic nematode of Djallonké sheep. *P. biglobosa* significantly reduced excretion of *H. contortus* eggs. Both plants were effective on the viability of adult *H. contortus* worms and significantly reduced the number of eggs per female worm showing their effect on female fertility. Their administration also contributed to the improvement of the hematocrit.

The use of these plants as an alternative to chemical anthelmintics is therefore promising. However, further investigations are needed to evaluate the toxicity of these plants and to understand their metabolism in the digestive tract of animals.

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