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The acute toxicity in mice and the *in vitro* anthelminthic effects on *Haemonchus contortus* of the extracts from three plants (*Cassia sieberiana*, *Guiera senegalensis* and *Sapium grahamii*) used in traditional medicine in Burkina Faso

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ABSTRACT

The acute toxicity in mice and the in vitro anthelminthic effects on Haemonchus contortus worms of aqueous extracts from three (3) plants: Cassia sieberiana D.C. (Caesalpiniaceae), Guiera senegalensis J.F Gmel. (Combretaceae) and Sapium grahamii (Hochst) Pax. (Euphorbiaceae) used in traditional medicine in Burkina Faso for the treatment of gastro – intestinal parasites are reported in this study. By oral route, the extracts of plants did not present toxicity on mice up to 2000 mg / kg in 72 h. By intra peritoneal way, the 50 % lethal doses (LD_{50}) were 273 mg / kg (leaves of Guiera senegalensis); 389 mg / kg (leaves of Sapium grahamii); 660 mg / kg (leaves of Cassia sieberiana) and 253 mg / kg (roots barks of Cassia sieberiana).The evaluation of the effects on Haemonchus contortus worms showed that at 0.1, 1, 3, 10 and 15 mg / mL, all the extracts provoked the death of the worms after 24 hours of contact. The mortality was dose dependent for the roots barks of Cassia sieberiana (ERCS) and the leaves of Sapium grahamii (FSG) and not dose-dependent for the leaves of Cassia sieberiana (FCS) and the leaves of Guiera senegalensis (FGS). The lethal concentrations (CL_{50}) were 0.8 ± 0.4, 0.39 ± 0.4, 0.17 ± 0.1 and 1.04 ± 0.7 mg/mL for ERCS, FCS, FGS and FSG respectively. These experimental results suggested that the extracts of the studied plants possess anthelminthic properties that would justify their use in traditional medicine against the gastro intestinal parasites.

Keywords: Medicinal plants, acute toxicity, anthelminthic, *in vitro*, Burkina Faso.

INTRODUCTION

The gastro intestinal parasites infections are diseases affecting all the countries of the world with ascendancy in the tropical zone. The WHO [1] estimated in 1994 at 2 billion, the number of persons who are chronically carriers of intestinal worms. These parasitic pathologies affect as well animals as humans in developing countries.

Helminthiases are more common in the tropics where poor hygienic conditions and poverty increase the risk of infection. In addition, these diseases are responsible for mortality in human beings and affect especially children of school age, thus, compromising their growth, intellectual, development and their school performance as well as increasing their vulnerability to other infections. On the other hand, in domesticated animals, helminthic infections impair health, welfare, and productivity. Worm infections result in increased death rate and poor growth and

reproduction [2]. Helminthiasis are the most wide-spread parasites infections and are responsible for the biggest economic losses of goats and sheeps in several regions in the world [3]. In developing countries a big proportion of the populations are moving towards to the ancestral knowledge on the plants for health care of humans and animals. Several medicinal plants were described in the African pharmacopoeia [4]. In Burkina Faso, the traditional medicine uses plants for the treatment of various diseases among which gastro intestinal parasites infections [5]; [6]. Surveys realized in four areas in Burkina Faso revealed that *Cassia sieberiana D.C* (Caesalpiniaceae), *Guiera senegalensis* J.F Gmel. (Combretaceae) and *Sapium grahamii* (Hochst) Pax. (Euphorbiaceae) are widely used in the traditional medicine against the gastro- intestinal parasites as well in humans as in livestock [7] Studies highlighted the antivenom, insecticidal and trypanocide properties of *Guiera senegalensis* [8]. According to Von Maydell [9], *Cassia sieberiana* is used in the abdominal pains, against the avian influenza, as laxative and as dewormer. The properties of *Sapium grahamii* against the Guinea worm were demonstrated by Gurib-Fakim [10]. In this study, we evaluated the acute toxicity of aqueous extracts of these three (3) plants and their *in vitro* anthelminthic effects on *Haemonchus contortus*, an abosomal nematode of sheep.

MATERIALS AND METHODS

-The Plants

The study was realized with the extracts of *Cassia sieberiana*, *Guiera senegalensis* and *Sapium grahamii*. The exploited parts are leaves and roots barks (table 1). They were collected around the city of Ouagadougou situated in north sudanian zone (zone of savanna) in Burkina Faso. Plants were identified at the herbarium of the Centre National de la Recherche Scientifique et Technologique (CNRST) in Ouagadougou where the specimens of the 3 plants are deposited under the voucher numbers HNBU00179; HNBU00252 and HNBU01032 respectively.

Table 1: The plant parts and the extracts used in the study

Plants (Family)	Parts used	Extract
Cassia sieberiana (Caesalpiniaceae)	leaves	FCS
Cassia sieberiana (Caesalpiniaceae)	roots barks	ERCS
Guiera senegalensis (Combretaceae)	leaves	FGS
Sapium grahamii (Euphorbiaceae)	leaves	FSG

-Preparation of Extracts

Leaves and barks were washed in water and dried shielded from the dust. Then, they were finely ground to realize decoctions and macerations according to the procedure of healers. Briefly, 100 g of vegetable powder were diluted in 1000 mL of distilled water and boiled during 1 hour, then cooled. Decoctions obtained were filtered on cotton wool then on the Whatman filter paper (2 V, 8 µm) before being freeze-dried and kept for the tests. For the macerations, 100 g of vegetable powder were dissolved in 1 000 mL of distilled water and let to macerate during 48 hours. The obtained solution is filtered on cotton wool then on Whatman filter paper (2 V, 8 µm). Extracts were freeze-dried and served for the various tests.

-The Animals

The animals used were NMRI mice provided by the Centre International de Recherche- Developpement de l'Elevage en Zone Subhumide (CIRDES) of Bobo Dioulasso (Burkina Faso). These mice were acclimatized to the conditions of the animal house of the Départment de Medicine et Pharmacopée Traditionnelle/Pharmacie (MEPHATRA/PH) of the Institut de Recherche en Sciences de la Santé (IRSS) of Ouagadougou (12:12 hour light/dark cycle, Temperature: 23-25°C, relative humidity about 75 %). They were fed with the food granules containing 29 % of proteins provided by the western regional office of the Centre de Promotion de l'Aviculture Villageoise (CPAVI) of Bobo-Dioulasso (Burkina Faso). The drinking water was supplied to them *ad libitum* throughout the experimental period.

-the experimental procedures

Acute toxicity

The NMRI mice, weighting between 25 and 35 g were put on a diet for 12 hours; they were allotted in 6 groups of six mice. Extracts were administrated by oral and intra peritoneal (IP) routes. The extracts were administered at 250, 500, 750, 1000 and 2000 mg/kg of body weight for group 1-5. The group 6 (control group) received the solvent (distilled water). Animals once treated were observed during the two hours which follow extracts administration and then they were fed. They were then observed after 24 h, 48 h and 72 h. Animal's intoxication symptoms were noted. The mice that died in each group were counted for lethal dose (LD_{50}) determination. The LD_{50} was estimated according to the initial method described by Trevan, [11] and its various and successive modifications: [12][13][14].

Anthelminthic effects on Haemonchus Contortus worms

The biological material was adult worms of *Haemonchus contortus* harvested on the stomach of naturally infected goats or sheep. The stomach were bought from the refrigerated slaughterhouse of Ouagadougou, conditioned in an icebox and forwarded in the laboratory. These organs were incised longitudinally with scissors to release the worms. The worms were carefully collected and placed in a Petri dish containing Phosphate Buffer Solution: (PBS, pH: 7.2). They were washed then successively in the PBS to be cleaned of fragments of faeces and then used immediately for the biological tests.

The test of inhibition of the motility of the worms described by Jabbar et al. [15] was used. The collected worms were put in Petri dish at the rate of 3 worms per box, in a total volume of 3 ml of PBS solution (negative control) and the extracts dissolved in the PBS at the increasing concentrations of 0.1; 1; 3; 10 and 15 mg / mL. The parasites were let to incubate at 37 °C during 24 hours. The motility and the survival of worms were observed using the optical microscope at 2 hours, 4 hours, 6 hours and 24 hours, after the exposure. Levamisole (1 % w:v) was used as positive reference substance.

Worms died after the exposure in extracts were counted at 4 hours, 6 hours and 24 hours and the mortality rate of worms (MR) for each concentration of extract was calculated by using the following formula:

MR (%) = (Number death worms / Number of worms put in the Petri dish) x 100.

The test was carried out in triplicate.

The average percentages of the mortality in worms were submitted to the analysis with the software Graph Pad Prism 5.0. All data were expressed as mean ± E.S.M. The various figures were drawn and the LC50 were determined by the same software. Student-Newman-Keuls *t*-test was used to determine significant differences between means. Mean values were considered significantly different when $P < 0.05$.

The lethality of mice was estimated as a percentage of deaths observed during the test for each dose of extract. The software Pharmacological Calculation System (PCS, version 10.0.5) was used to determine the values of LD1, LD50, and LD99 and the relative ratios LD50/LD1, LD99/LD1 and LD99/LD50. The scale of Hodge and Sterner [16] was used to characterize the safety level of each plant extract.

RESULTS

-Oral toxicity of extracts

The various extracts did not provoke mortality in mice up to the dose of 2000 mg / kg within 72 hours (Table 2).
For the extract of *Guiera senegalensis* (FGS), no distinguishing sign of toxicity was noted.
A reduction in the motricity activity was observed for the roots and leaves extracts of *Cassia sieberiana* (FCS) whereas for the leaves extract of *Sapium grahamii* (FSG), an increase of the motricity activity and an aggressiveness of the animals were observed.

-IP toxicity of extracts

During the 72 hours which followed the administration of extracts, an increase of the motricity
activity, the twisting of the back train, convulsions were observed in mice treated with the extract of *Sapium grahamii*. A slumber during the first hours after administration of the extracts of *Cassia sieberiana* (FCS and ERCS) was observed in animals treated with this plant. The mortality was noticed at 72 hours after the injection of FCS, ERCS and FGS. For the FSG, the mortality occurred 24 hours after the administration.

The DL_{50} of extracts were 273 mg/kg; 389 mg/kg; 660 mg/kg and 253 mg/kg for the leaves extract of *Guiera senegalensis* (FGS), leaves extract of *Sapium grahamii* (FSG), the leaves extract of *Cassia sieberiana* (FCS) and the roots bark extract of *Cassia sieberiana* (ERCS) respectively (Table 2).

Table 2: The LD_{50} values of the extracts administrated by intra-peritoneal (IP) and oral routes in mice

Extracts	IP route		Oral route	
	LD_{50} (mg/kg)	Delay (hours)	LD_{50} (mg/kg)	Delay (hours)
ERCS	253	72	>2000	72
FCS	660	72	>2000	72
FGS	273	72	>2000	72
FSG	389	24	>2000	72

ERCS= Roots bark extract of Cassia sieberiana, FCS = Leaves extract of Cassia sieberiana , FGS= Leaves extract of Guiera senegalensis, FSG = Leaves extract of Sapium Grahamii.

Table 3: The LD_{50} values (mg/kg) and the toxicological parameters for the extracts of *Cassia sieberiana, Guiera senegalensis* and *Sapium grahamii* by intra-peritoneal (IP) route in mice

Extracts	LD_{99} (mg/kg)	**LD_{50}**(mg/kg)	LD_1(mg/kg)	LD_{99}/LD_{50}	LD_{50}/LD_1	LD_{99}/LD_1
ERCS	1338. 8	**253**	47.8	5.3	5.3	28
FCS	1747.7	**660**	249.5	2.65	2.65	7.0
FGS	945	**273**	78.8	3.5	3.5	11.9
FSG	2332	**389**	64.9	6	6	35.9

-Effects on Haemonchus Contortus worms

After 24 hours of incubation, the percentages of mortality of *H. contortus* worms were estimated for every concentration of the extracts of plants tested.

The percentages of mortality of worms at the concentration of 15 mg/ml were 62.67 ± 6; 81.34 ± 8.6; 96 ±3 and 85 ± 3.3 for ERCS, FCS, FGS and FSG respectively (Figure 1). The percentage of mortality was 100 % a 24 hours for the Levamisole (1 % w: v). Compared with the average percentage noted with the PBS (11 ± 0.6), the effects of the Levamisole and the extracts were significantly different ($p < 0.05$).

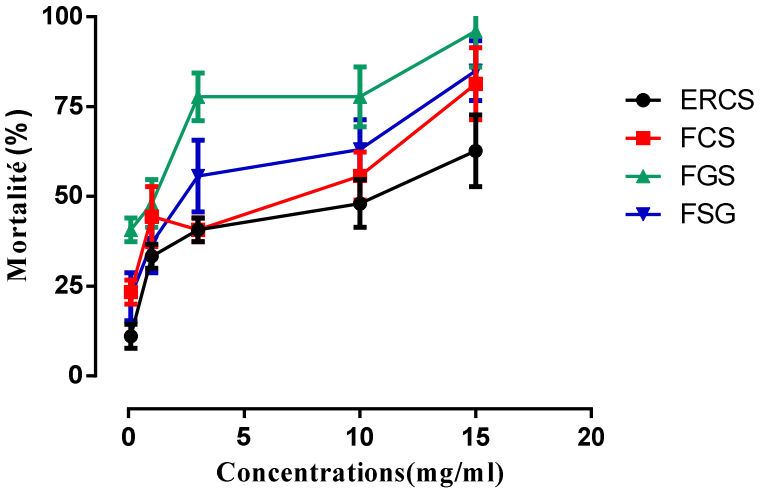

Figure 1 : Dose-response profile for mortality (in percentage) of adult worms of *Haemonchus contortus* by increased concentrations of the plants extracts after 24 h incubation (n=9)

Table 4: The lethal concentration 50 (LC_{50}) values (mg/ml) of the three plants' extracts against *Haemonchus contortus*

Extracts	LC_{50} (mg/mL)	Maximal lethality (%)
ERCS	0.80 ± 0.4	63 ± 6.0
FCS	0.39 ± 0.4	81.3 ± 11.6
FGS	0.17 ± 0.1	96.0 ± 10.0
FSG	1.04 ± 0.7	85.0 ± 11.6

DISCUSSION

The study of the toxicity of a drug used in traditional medicine is of major importance to determine its harmlessness with the aim of a use without risk of poisoning. In our study, for the extracts administrated by oral route, no mortality was observed up to the dose of 2000 mg/kg. It was concluded that the LD_{50}'s of the extracts of *Cassia sieberiana*, *Guiera senegalensis* and *Sapium grahamii* were higher than 2000 mg/kg by the oral route. These extracts could be classified as slightly toxic according to the Hodge and Sterner scale [16] and in the class II (slight dangerous) on the WHO scale [17]. By intra peritoneal route, the LD_{50} were 273 mg/kg; 389 mg /kg; 660 mg/kg; 253 mg/kg; for the leaves of *Guiera senegalensis*, the leaves of *Sapium grahamii*, the leaves of *Cassia sieberiana* and the root bark of *Cassia sieberiana* respectively. These values indicated that such drugs could be classified in the groups of weakly toxic (FCS) or moderately toxic substances (ERCS, FGS and FSG) according to the HODGE and STERNER scale [16]. On another hand, they could be classified in the class III of the WHO scale [17].

Our results on stem bark of *Cassia sieberiana* are comparable to those of Fane [18] who obtained for the stem bark extract of the same plant a LD_{50} of 400 mg/kg by IP route in mice. *Cassia sieberiana* is well known in several regions of western Africa, from Senegal to Nigeria, as a toxic plant used as hunting poison [19]. Tamboura et al. [20] obtained for the root bark of the same plant a LD_{50} of 24,4 mg/kg by IP route in mice; so concluding that the plant would be very toxic. Our results are different of those of these authors. This difference could be understandable by the edaphic factors. Indeed, Adjanohoun et al. [21] reported that *Cassia sieberiana* growing on some substrates, in particular termitaries presents a very high toxicity.

The weak toxicity of *Guiera senegalensis* has been reported in Nigeria by Abubakar et al [22] who determined a LD_{50} of 1300 mg /kg by IP way in mice. Our results indicated a higher toxicity compared to that determined by these authors.

Sapium Grahamii is considered very toxic and used as poison of arrows in Ivory Coast and for ritual scarification by the Hausas peoples of Nigeria [10]. Nevertheless, the index of security given by the ratio LD_{99}/LD_1 equal to 7.0 (Table 3) for the leaves extract of *Cassia sieberiana*; suggests the necessity of a precaution in using this plant. This index is greater than 10 for the other extracts, showing their good handiness of use. The ratios of LD_{50}/LD_1 and LD_{99}/LD_1 are quite equal for the plants studied, confirming the validity of our LD_{50} determination test [23]. In the treated groups the mice death was reported within 12 hours for the extract of *Sapium grahamii* and within 72 hours for the extracts of *Guiera senegalensis* and *Cassia sieberiana*. This suggests an immediate toxicity for *Sapium grahamii* and a delayed toxicity for *Guiera senegalensis* and *Cassia sieberiana*.

The animals presented some signs of toxicity; a reduction in the motricity activity is observed for ERCS. For FSG, an increase of this activity and aggressiveness were observed in animals. The slumber and the aggressiveness observed to mice could be due to the action of chemical compounds contained in extracts and which would act on the inhibitory and excitatory functions of the central nervous system. Indeed, it was demonstrated that compounds such as the terpenic compounds possessed sedative properties [24].

The results of the study on the mortality of worms showed that extracts presented anthelminthic properties on the adult forms of *H. contortus*. The CL_{50} values for the extracts (Table 4) showed that FGS and FCS were more effective than FSG and ERCS. Similar results were reported on this parasite [25]. Some mechanisms which underline the anthelminthic activity on nematodes were described by several authors: the distribution of anthelminthic through the external surface of the cuticles of the parasites and the distribution in the intestinal cells of the host [26]. By these mechanisms, the active molecules of plants would act by binding the proteins, so inducing the death by paralysis of the parasites [27]. The levamisole used as reference acts as a selective agonist of the nicotinic receptors of acetylcholine. It induces the contraction of the somatic muscles of the nematode which leads to its death [28]. The tested plants extracts could act according to this modality.

CONCLUSION

The results of the present study showed that the used extracts presented a weak or moderate toxicity. The index of security indicates a good handiness, what is beneficial for the user. The *in vitro* evaluation of the effects on the adult worms of *Haemonchus contortus* revealed that the extracts would possess an anthelminthic effect. This would justify the use of the studied plants in traditional medicine in Burkina Faso for the treatment of gastro-intestinal parasites in human and animal. Nevertheless, there is the need to carry out experimental to study the phytochemical profile and to assess the mechanisms underlying the anthelmintic effects of these plants.

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