Journal of Intercultural Ethnopharmacology
www.jicep.com
DOI: 10.5455/jice.20170810024110

Original Research

Anthelmintic effects and toxicity of *Cynodon dactylon* (L.) Pers. in rodent models

Arun K. Yadav, Purobi Nath

Department of Zoology, North-Eastern Hill University, Shillong, Meghalaya, India

Address for correspondence:
Arun K. Yadav, North-Eastern Hill University, Shillong - 793 022, Meghalaya, India.
E-mail: akynehu@hotmail.com

Received: August 10, 2017
Accepted: October 15, 2017
Published: November 23, 2017

ABSTRACT

Background: *Cynodon dactylon* (L.) Pers. is a very widely distributed medicinal herb. Different preparations from this herb are extensively used as a folk medicine in the Middle East, including Turkey, besides in India, Italy, etc. In India, the grinded juice of *C. dactylon* has been commonly used as a folk medicine for the treatment of intestinal-worm infections. **Aim:** This study was undertaken to investigate the *in vitro* and *in vivo* anthelmintic effects and potential toxicity of whole plant extract of *C. dactylon* against *Hymenolepis diminuta* (hymenolepididae), a zoonotic tapeworm, using two rodent models. **Materials and Methods:** In *in vitro* assay, plant extract was tested at 10, 20, and 40 mg/ml concentrations, against adult *H. diminuta*, and the efficacy was adjudged by physical motility and mortality of parasites. *In vivo* testing was done in *H. diminuta* - Wistar rat model, by monitoring the eggs per gram of feces (EPG) count and worm counts of animals after treatment with 200, 400, and 800 mg/kg doses of extract. Acute toxicity of extract was monitored with 2000 mg/kg oral dose of extract, following the Organization for Economic Cooperation and Development (OECD) guidelines in Swiss albino mice. In subacute toxicity, a low extract dose, i.e., 400 mg/kg and a high extract dose, i.e., 800 mg/kg was tested in mice, as per the guidelines of the OECD, for the assessment of some hematological and biochemical parameters of mice. **Results:** The extract showed a dose-dependent efficacy in both, the *in vitro* assay as well as in the *in vivo* assay. In the *in vitro* test, the 40 mg/ml concentration of extract caused paralysis and mortality of worms in as early as in 4.12 ± 0.55 h and 5.16 ± 0.34 h, respectively. This was comparable with the reference drug praziquantel (PZQ). The *in vitro* anthelmintic effects were also corroborated by the results of *in vivo* assay, wherein treatment of rats with 800 mg/kg dose of extract for 5 days revealed up to 77.64% reduction in EPG counts and 79.00% reduction in worm counts at post-treatment period, showing a comparable efficacy with 5 mg/kg dose treatment of PZQ. In the acute toxicity assay, the extract did not reveal any adverse effects or mortality in any animal, during the 14-day observation period. The LD_{50} of extract was estimated to be greater than 2000 mg/kg. In the subacute toxicity study, all the studied parameters of animals were found to be normal at 400 mg/kg dose (low dose); however, treatment with high dose, i.e., 800 mg/kg revealed only a slight elevation of aspartate aminotransferase in animals. **Conclusion:** *C. dactylon* possesses significant anthelmintic properties, and its extract appears to be devoid of any major adverse effects in experimental animals. These pharmacological credentials support the safe folkloristic use of this plant as an anthelmintic remedy.

KEY WORDS: Anthelmintic, *Cynodon dactylon*, folklore medicine, helminths, *Hymenolepis diminuta*, India, middle east, Poaceae, soil-transmitted helminths, traditional medicine

INTRODUCTION

Intestinal helminthic infections are among the most common infections worldwide and affect the poorest and most deprived communities. They are widely distributed in all World Health Organization (WHO) regions, and in particular, in many parts of Asia and Africa [1]. In endemic areas, the WHO recommends periodic treatment with anthelminthic medicines, such as albendazole and mebendazole. However, in many parts of the world, especially in India, people also use traditional medicines to cure helminthic infections [2]. Therefore, nowadays, there is a renewed interest in medicinal plant research.

India with a large population and ethnic diversity possesses a rich legacy of many traditional medicine practices. While some of its traditional medicines are well documented, for example, Ayurveda, Unani, Homeopathy, or Siddha medicines, other practices, such as tribal folk medicines, still lack a systematic documentation or proper scientific validation [2]. Most tribal folk medicines in India are usually focal in nature and are practised in small tribal settlements,

which are often located in remote and rural areas of the country [2]. The Northeast region of India, in particular, is unique because it is a home of more than one hundred indigenous tribes. Each tribe in this region possesses its own know-how about the use of various folk medicines to cure their day-to-day common health problems [2]. Reang tribe constitutes as one of the major tribes of Northeast India, which are mainly distributed in North Tripura, Dhalai, and South Tripura districts of Tripura state, besides in some parts of Assam and Mizoram states and Bangladesh [3]. During our recent ethnopharmacology studies of Reang tribes in Tripura state, we came across a few medicinal plants which are frequently used as an anthelmintic remedy by the people of this tribe [3].

Cynodon dactylon (L.) Pers. (Bermuda grass), (Family: Poaceae), is a very widely distributed perennial medicinal herb [4]. It is commonly found in Turkey, Iran, Afghanistan, India, and Pakistan [5]. A recent ethnopharmacological field study, involving 400 local respondents, in Tripura state of India revealed that *C. dactylon* is one of the popular medicinal herbs used for the treatment of intestinal-worm infections [2]. This survey also revealed that about 81% of local residents have utilized this herb to treat the intestinal worm infections [2]. The literature survey revealed that *C. dactylon* is highly praised for its various beneficial biological effects in different regions of the world, especially in India and in some Middle East countries. In Indian Ayurvedic system of medicine, *C. dactylon* is used for the treatment of diarrhea, vomiting, burning sensation, fever, and skin diseases [4]. On the other hand, the Santhal tribes of Assam and West Bengal states of India apply a paste prepared from this plant as a remedy for a headache [6]. In the Unani system of medicine, *C. dactylon* is used as a laxative, coolant, expectorant, carminative agents. [7]. Elsewhere, in the Tyrrhenian part of the Basilicata region of southern Italy, a decoction and a tablet prepared from *C. dactylon* have also been employed against malaria and kidney stone problems [8]. On the other hand, in the Meriç Town region of Turkey, the root and whole plant decoction of this herb are drunk as a cure for prostate ailments and rheumatism kidney stones [9]. In the same country, in the northwest Anatolia areas, a cooled decoction from *C. dactylon* is also drunk for the treatment of gonorrhea [10]. Some *in vitro* and *in vivo* studies on this plant reveal that it possesses antioxidant, antidiabetic, antimicrobial, hepatoprotective, wound healing, and antiarthritic properties [11-17]. *C. dactylon* has been reported to possess many compounds, which include some major secondary metabolites, such as flavonoids, alkaloids, glycosides, terpenoids, besides glycerin, 9, 12-octadecadienoyl chloride, (Z, Z), hexadecanoic acid, ethyl ester, ethyl α-D-glucopyranoside, linoleic acid, ethyl ester, β-sitosterol, leachianol G, leachianol F, and phytol [18-20].

However, there are only limited studies related to the anthelmintic potentials of this plant. Further, due to one reason or other, from the existing few studies on this plant, it is also quite difficult to draw a conclusive evidence about the anthelmintic potentials of this herb. For example, in one study, the *in vitro* anthelmintic effects of *C. dactylon* were investigated using earthworm, *Pheretima posthuma* as a test parasite [21]. However, the findings of this study does not seem to hold much authentic scientific evidence, because in this study, the experiments were conducted using free-living organisms, earthworms as test organisms, which except for some morphological or anatomical resemblance, do not possess all the physiological attributes possessed by an intestinal helminth-parasite [21]. Likewise, two other field studies attempted to assess the anthelmintic potentials of this plant in goats infected with gastrointestinal nematodes (GIN) [22,23]. These studies tried to monitor the fecal egg count reductions of GIN in goats, following their grazing on control pastures with forage paddocks of *C. dactylon* and *Lespedeza cuneata*, separately, and also the paddocks with the combinations of these two forages together [22,23]. The results of these studies revealed that grazing of animals on pastures having a combination of these two plants together had comparatively lower worm burdens than those goats which graze on pastures of *C. dactylon* alone. In the light of these ambiguous facts, in the present study, we were interested to systematically investigate the anthelmintic effects of *C. dactylon*, using both, an *in vitro* assay as well as an experimental *in vivo* model of a zoonotic intestinal cestode, *Hymenolepis diminuta* (hymenolepididae) that was maintained in the albino rat. This host-parasite model has been recognized as a suitable model for screening anticestodal drugs [24]. Furthermore, in this study, we addressed the acute and subacute toxicity potentials of this plant, using some selected biochemical and hematological parameters in Swiss albino mice.

MATERIALS AND METHODS

Plant Material

The plant material [Figure 1] for this study was collected from North Tripura district of Tripura (24° 36' N latitude and 92° 19' E longitude) and was duly authenticated by a taxonomist in the Department of Botany, North-Eastern Hill University (NEHU), Shillong. A voucher specimen (No. AKY- 11881) has been retained in the Department of Zoology, NEHU. The whole plant material was dried under shade and powdered for extraction with methanol in a Soxhlet extractor at 40°C. The extract was reduced to dryness using a rotary evaporator and stored at +8°C until use. The final yield of methanol extract was 15% (w/w).

Experimental Animals

For anthelmintic testing, male and female albino rats of Wistar strain, weighing 180-200 g, were used, while for toxicological experiments, Swiss albino mice, 25-30 g, of either sex were utilized. Before use in experiments, all the animals were acclimatized for 15 days in the laboratory and had *ad libitum* access to standard rodent food and water. They were housed individually in acrylic cages. All the experiments on these animals were performed after the due approval of the Institutional Ethics Committee (Animal Models) of NEHU, Shillong.

Anthelmintic Studies

Maintenance of animal model

For *in vivo* experiments, the lifecycle of *H. diminuta* was maintained in the laboratory by alternating the hosts, Wistar rats, and Flour beetles [25].

In vitro anthelmintic assay

Live adult *H. diminuta* specimens were obtained by performing the necropsy of laboratory albino rats, carrying induced infections of this parasite. For each *in vitro* assay, six live specimens of parasite were placed in a Petridish, containing 10 ml of Hank's solution (pH 7.3), and maintained inside an incubator at 37 ± 1°C. The extract and reference drug, praziquantel (PZQ), was dissolved in a few drops of 1% dimethyl sulfoxide (DMSO). Plant extract was tested at 10, 20, and 40 mg/ml concentrations, whereas PZQ at 1 mg/ml concentration. An additional Petridish, containing an equal amount of 1% DMSO and six parasite specimens, was also included to serve as control. The *in vitro* anthelmintic efficacy was adjudged in terms of physical motility of worms, as evidenced by paralysis and mortality of test parasites [26].

In vivo assay

The extract was tested against adult *H. diminuta*, maintained in albino rats. Animals were divided into five groups, each consisting of six animals. Each animal was orally inoculated with 4 cysticercoids by a blunt feeding tube and maintained in a separate cage. Group I of animals served as infected, untreated control and were given 1.0 ml of saline, plus few drops of 1% DMSO (vehicle), daily on days 21-25 post-inoculation (p.i.) of cysticercoids. Groups II to IV of animals were treated with 200, 400, and 800 mg/kg single doses of extract that were given between days 21-25 p.i. of cysticercoids. Group V of animals was given a reference drug, PZQ (in 1% DMSO) that was given during the day 21-25 p.i. of cysticercoids. The EPG counts of experimental animals were undertaken for 3 days (day 18-20) before treatment (pre-treatment EPG) and for 3 days (day 26-28) after extract treatment (post-treatment EPG) by examining the fecal pellets, collected from each animal cage, and reductions in EPG counts were worked out using the modified McMaster method [27]. Finally, all the experimental animals were sacrificed on day 39, and the worms from their intestine were collected to calculate the percentage reduction in worm counts.

Toxicity Studies

The acute toxicity of extract was performed according to the procedures of Organization for Economic Cooperation and Development (OECD) revised limit dose test for acute toxicity testing [28]. All the animals were fasted overnight before the administration of extract. Later, each animal was dosed individually with 2000 mg/kg dose of extract and observed carefully for any adverse toxicity or mortality for 14 days. The LD_{50} was predicted to be above 2000 mg/kg if three or more animals survived in this experiment.

The subacute toxicity of extract was performed as per the OECD guidelines 427 [29]. Based on the findings of the acute toxicity test, two different doses of extract, i.e., 400 mg/kg (low dose) and 800 mg/kg (high dose), were selected and administrated orally daily for 14 days to two different groups of mice (n = 10). The third group of mice (n = 10) was included as a control and received only vehicle for the same duration [30]. After extract treatment, all the experimental animals were carefully observed daily for any abnormal clinical signs and mortality for 14 days. At the end of 14-day observation period, all the animals were anesthetized, and their blood samples were collected with and without anticoagulant (ethylenediaminetetraacetic acid), for hematological and biochemical studies, respectively. In hematological analysis, red blood cell, white blood cell, and platelet counts, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were analyzed, using a hematology analyzer (Nihon Kohden Celltac MEK 6410 K Cell Counter). In biochemical analysis, blood without additive was centrifuged at 3000 × g at 4°C for 10 min, serum was separated, and levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, total bilirubin, direct and indirect bilirubin, urea, and creatinine were evaluated, using a semi-automated Biochemical Analyzer (Bayer RA-50).

Statistical Analysis

Data are expressed as mean ± standard errors of the mean. Evaluations were performed using Student's t-test, and by one-way analysis of variance, followed by Bonferroni test. $P < 0.05$ was considered as statistically significant.

RESULTS

Anthelmintic Efficacy

In vitro exposure of *H. diminuta* worms to 10, 20, and 40 mg/ml concentrations of extract revealed a dose-dependent efficacy [Figure 1]. At 40 mg/ml concentration, the parasites showed paralysis in 4.12 ± 0.55 h, which was followed by their mortality in 5.16 ± 0.34 h. In comparison, the reference drug, PZQ (1 mg/ml), showed paralysis and mortality of worms at 0.34 ± 0.06 h and 0.89 ± 0.31 h, respectively. The plant extract exhibited significant decrease ($P < 0.001$) in the mortality time of worms when compared to the untreated control. The worms maintained in the control medium showed physical activity till 20.13 ± 3.02 h [Figure 2].

The *in vivo* testing of the extract against adult *H. diminuta* infections in rats also showed a dose-dependent efficacy [Table 1]. Treatment of rats with 800 mg/kg dose of extract for 5 days revealed 77.64% reduction in EPG counts and 79.00% reduction in worm counts at post-treatment period.

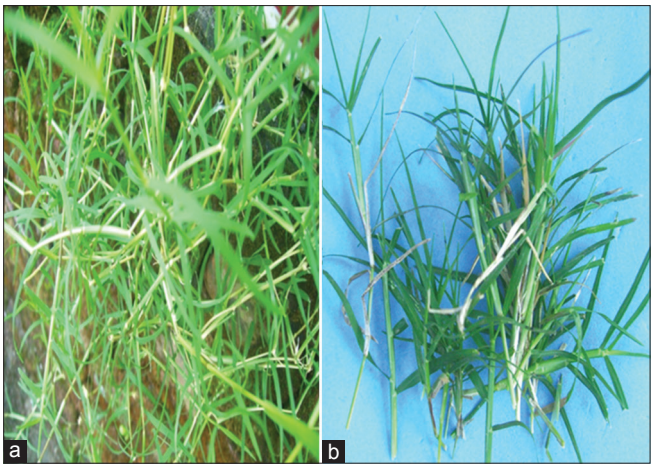

Figure 1: *Cynodon dactylon,* (a) whole plant in its natural habitat, (b) enlarged view of leaves

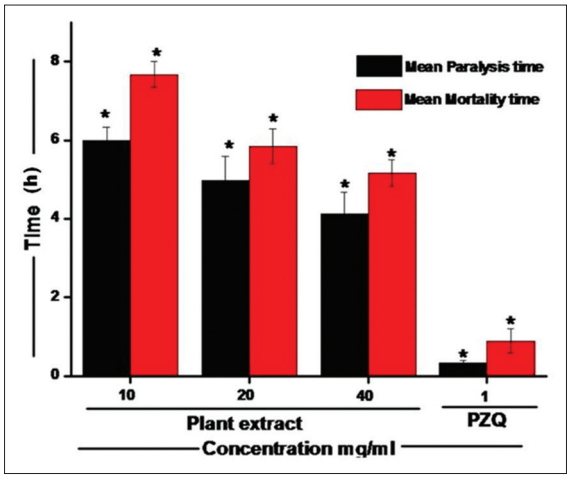

Figure 2: *In vitro* anthelmintic effects of *Cynodon dactylon* extract against *Hymenolepis diminuta*. The physical activity of test worms ($n = 6$) in the control group was recorded to be 20.13 ± 3.02 h. *$P < 0.001$ as compared to control group

Toxicity Studies

In acute toxicity study, administration of 2000 mg/kg dose of extract to five mice did not reveal any adverse signs or mortality up to 14 days observation period. Therefore, the oral LD_{50} of the extract was estimated to be greater than 2000 mg/kg in mice.

The effects of subchronic administration of *C. dactylon* extract on biochemical parameters of mice are presented in Table 2. In general, except AST, no other analyzed biochemical parameter showed any significant difference between the extract-treated and control groups. The AST showed only slight elevation ($P < 0.05$) in the high-dose extract (800 mg/kg)-treated group. Although somewhat moderate elevation was also noticed in the levels of ALT in high dose-treated group, the elevation was not statistically significant when compared with the control. In hematology analysis, none of the studied parameters showed any noticeable changes in the extract-treated animals [Table 3].

DISCUSSION

India has a very rich and ancient tradition of using different traditional medicines [31]. In its northeast region, in particular, which is densely inhabited by several indigenous tribes, the use of herbal folk medicines is very common practice [2]. However, given the magnitude of their use by common people, there has not been enough attention paid to scientifically validate acclaimed therapeutic effects, and more importantly, the risk associated with the use of these folk medicines. In the recent times, however, much emphasis is being given for the evidence-based ethnopharmacological use of medicinal plants [31]. Therefore, in the present study, we were mainly interested to scientifically investigate the *in vitro* as well as *in vivo* anthelmintic effects of *C. dactylon*, which was documented to be among one of the most widely used anthelmintic medicinal plants in Northeast region of India. In addition, the acute and subacute toxicity profile of this plant has also been investigated, employing some biochemical and hematological parameters in albino mice.

In most previous bioprospecting studies, the scientific validations of medicinal plants have mainly been done using different *in vitro* assays [25,32]. However, there are only few studies where folk medicines have been systematically studied using both, the *in vitro* as well as *in vivo* experimentations, besides any remarks on the safety profiles of medicinal plants [25,32]. In the present study, the whole plant extract of *C. dactylon* showed the significant and dose-dependent effect on paralysis and mortality of adult *H. diminuta* parasites. At 40 mg/ml concentration, the parasites showed paralysis in 4.12 ± 0.55 h, which was followed by their mortality in 5.16 ± 0.34 h. In comparison, the reference drug, PZQ (1 mg/ml), showed paralysis and mortality of worms at 0.34 ± 0.06 hand 0.89 ± 0.31 h, respectively. This finding was further corroborated by the results of *in vivo* testing of its extract against the same parasite in rats. In *in vivo* assay, the extract also showed significant anthelmintic effects as was evident by a significant decrease in the eggs per gram of feces (EPG) count and worm counts of animals following treatment with different doses of extract. Treatment of rats with 800 mg/kg dose of extract for 5 days revealed 77.64% reduction in EPG counts and 79.00% reduction in worm counts at post-treatment period. In comparison, treatment with PZQ (5 mg/kg) for the same duration revealed 71.88% reduction in EPG counts and 75.00% reduction in worm counts at post-treatment period.

In a related *in vitro* study, the anthelmintic effects of *C. dactylon* were studied against the GIN of goats [33]. However, the concentration of extract used for testing in the *in vitro* assay was 100 mg/ml in this study, which seems a considerably higher concentration for *in vitro* assay. Further, in this study, the 100 mg/ml concentration of extract has been reported to kill only 50% GIN of goats, as against 100% cestode mortality by 30 mg/ml extract concentration within about 5 h noted in the present study. More importantly, this study also lacked any positive and/or negative controls in extract testing, which seems a major experimental drawback [33]. In a similar manner, another investigator also claimed that *C. dactylon* possesses good *in vitro*

Table 1: Anthelmintic effects of *Cynodon dactylon* extract* on adult *H. diminuta* worms in rats

Group	EPG counts (mean±SEM)		Percentage difference in EPG counts (A-B)	Number of worms recovered/rat (mean±SEM)	Percentage reduction in worm counts
	Pre-treatment Days 18–20 (A)	Post-treatment Days 26–28 (B)			
Control	16460±186	16911±210	+2.74	3.84±0.17	4.00
Plant extract					
200 mg/kg	16844±166	8711±201**	−48.28	1.84±0.34***	54.00
400 mg/kg	17153±232	5088±187**	−70.34	1.00±0.00***	75.00
800 mg/kg	17286±201	3866±221**	−77.64	0.84±0.31***	79.00
Praziquantel					
5 mg/kg	16200±231	4555±186**	−71.88	1.00±0.37***	75.00

Values are presented as the mean±SEM, $n=5$; *Administration of plant extract and praziquantel on days 21-25 post-inoculation with four cysticercoids per rat; **$P<0.001$ as compared to pre-treatment EPG value, one-way ANOVA *post-hoc* Bonferroni test; ***$P<0.001$ as compared to EPG in control group, one-way ANOVA *post-hoc* Bonferroni test. SEM: Standard error of the mean, ANOVA: Analysis of variance, EPG: Eggs per gram, *H. diminuta: Hymenolepis diminuta*

Table 2: Effects of subacute oral administration of *Cynodon dactylon* extract on selected biochemical parameters of mice

Parameters	Group I Control	Extract treatment for 2 weeks	
		Group II 400 mg/kg	Group III 800 mg/kg
AST (U/I)	93.63±0.04	96.01±0.14	112.16±0.8*
ALT (U/I)	52.15±1.16	62.0±1.10	60.01±0.63
SALP (U/I)	149.31±0.32	154.00±0.60	156.00±1.20
Total bilirubin (mg/dl)	1.00±0.02	0.50±0.60*	0.90±0.17
Direct bilirubin (mg/dl)	0.19±0.05	0.25±0.19	0.21±0.21
Indirect bilirubin (mg/dl)	0.39±0.03	0.40±0.50	0.41±0.20
Urea (mg/dl)	14.45±0.04	14.49±0.21	15.51±0.19
Creatinine (mg/dl)	0.52±0.04	0.50±0.20	0.59±1.70

Values are presented as the mean±SEM, $n=10$). *$P<0.05$ as compared to control, one-way ANOVA *post-hoc* Bonferroni test. SEM: Standard error of the mean, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase

Table 3: Effects of sub-acute oral administration of *Cynodon dactylon* extract on selected hematological parameters of mice

Parameters	Group I Control	Extract treatment for 2 weeks	
		Group II 400 mg/kg	Group III 800 mg/kg
RBC count ($\times10^6$ mm^{-3})	4.00±0.05	4.01±0.44	4.16±0.51
WBC count ($\times10^6$ mm^{-3})	5.50±0.31	5.16±0.05	5.85±0.30
Neutrophils (%)	46.6±0.30	47.2±0.54	47.6±0.21
Lymphocytes (%)	51.40±0.67	50.4±0.17	51.09±0.22
Monocytes (%)	2.10±0.30	2.60±0.31*	2.30±0.05
Hemoglobin (g/dL)	10.41±1.21	11.23±1.08	11.09±1.25
Mean corpuscular Hb (pg)	28.67±0.21	30.89±0.67	29.31±0.31
Mean corpuscular Hb conc (g/dl)	30.02±0.30	31.33±0.27	30.75±0.44
Mean corpuscular volume (fl)	85.61±0.19	84.22±0.21	86.13±0.29
Platelet count ($\times10^3$ mm^{-3})	1.90±0.10	2.00±0.09	2.10±0.05
PCV (%)	29.00±0.44	31.29±0.67	30.08±0.30

Values are presented as the mean±SEM, $n=10$. *$P<0.05$ as compared to control, one-way ANOVA *post-hoc* Bonferroni test. SEM: Standard error of the mean, RBC: Red blood cell, WBC: White blood cell, PCV: Packed cell volume

anthelmintic efficacy [21]. However, this study also appears to possess some drawbacks. First, instead of using a parasitic worm as test organism, these investigators used free-living earthworm as test parasites, which are considered physiologically different from parasitic helminths. Second, at 50 mg/ml concentration of *C. dactylon* extract, the time taken for mortality of earthworm in this study was about 48 h, as compared to about only 5 h recorded in our study [21].

The present findings of *in vivo* anthelmintic efficacy of *C. dactylon* extract also gains support from the results of some previous related *in vivo* efficacy of this plant undertaken in other hosts [22,23]. For example, two other studies attempted to establish the *in vivo* anthelmintic efficacy of this plant on the basis of reduction in the fecal egg count reductions of GIN of goats following their pasture grazing on forage paddocks of *C. dactylon* and *L. cuneata*, separately, and also the paddocks with combinations of these two forages together [22,23]. In these studies, also *C. dactylon* was noticed to possess a moderate degree of anthelmintic efficacy. β-sitosterol, flavonoids, alkaloids, glycosides, and terpenoids have previously been reported as some major compounds present in *C. dactylon* [18]. It is possible that one or more of these constituents may be responsible for the anthelmintic effect of this plant, as there are several published studies which report these chemical constituents to possess anthelmintic properties [34,35].

In the toxicity assessment, treatment of mice with 2000 mg/kg dose of extract did not reveal any evidence of adverse effect or mortality in any animal. Hence, the oral LD_{50} value of extract may be interpreted to be higher than 2000 mg/kg, indicating that the extract is practically non-toxic in mice, as per the criterion of the OECD guidelines. This finding is in agreement with a related study in which the acute toxicity of *Salvia scutellarioides* extract was investigated in mice, using the up and down procedure of the OECD guidelines [36]. In the latter study also, treatment with 2000 mg/kg dose of *S. scutellarioides* extract did neither reveal any change in the general appearance of experimental animals nor does it caused mortality of animals up to the observation period of 14 days [36].

In the subacute treatment of animals, except AST, no other analyzed biochemical or hematological parameters showed

any significant differences in the treated animals. The AST showed only slight elevation in the high dose (800 mg/kg) treated group of animals. Liver function tests are generally monitored by taking into account the serum levels of ALT and AST, besides few other enzymes. The ALT is found in a very high concentration in the liver [37] and considered to be an important indicator of hepatocellular damage. In the present study, the extract administration did not reveal any effects on the levels of ALT. AST is regarded to be less liver-specific than ALT, as an indicator of liver function, because it is also found in several other tissues, such as heart, lungs, skeletal muscle, and kidney. [38]. In many cases, any drug-induced mild elevation of AST has been found to normalize in a few days after discontinuing the drug.

CONCLUSION

The findings of the present study prove that whole plant extract of *C. dactylon* possesses significant anthelmintic properties, and as such, it does not show any major adverse effects in experimental animals. These pharmacological credentials support the safe folkloristic use of this plant as an anthelmintic remedy.

ACKNOWLEDGMENTS

PN was recipient of a Research Fellowship in Science for Meritorious Students by the University Grants Commission, New Delhi. We would like to thank the two anonymous reviewers for their suggestions and Mr. Santosh Vishwakarma, BIC, NEHU, for assistance in figure preparations.

REFERENCES

1. Intestinal Worms. World Health Organization. Available from: http://www.who.int/intestinal_worms/more/en. [Last accessed on 2017 Oct 12].
2. Tandon V, Yadav AK, Roy B, Das B. Phytochemicals as cure of worm infections in traditional medicine systems. In: Srivastava UC, Kumar S, editors. Emerging Trends in Zoology. New Delhi: Narendra Publishing House; 2011. p. 351-78.
3. Nath P. Evaluation of Anthelmintic Activity of Some Medicinal Plants Used in the Folk-Lore Medicine System of Riang Tribe in Tripura, Ph.D. Thesis, Department of Zoology. Shillong: North Eastern Hill University; 2014. p. 284.
4. Asthana A, Kumar A, Gangwar S, Dora J. Pharmacological perspectives of *Cynodon dactylon*. Res J Pharm Biol Chem Soc 2012;3:1135-47.
5. Harlan JR, de Wet JM. Sources of variation in *Cynodon dactylon* (L.) Pers. Crop Sci 1969;9:774-8.
6. Bodding PO, editor. List of santal prescription. In: Studies in Santal Medicine and Connected Folklore. Calcutta: The Asiatic Society; 1986. p. 162-3.
7. Nagori BP, Solanki R. *Cynodon dactylon* (L.) Pers.: A valuable medicinal plant. Res J Med Plant 2011;5:508-14.
8. Guarrera PM, Salerno G, Caneva G. Folk phytotherapeutical plants from Maratea area (Basilicata, Italy). J Ethnopharmacol 2005;99:367-78.
9. Kartal C, Güneş F. Medicinal plants used in Meriç town from Turkey. Indian J Pharm Educ 2017;51:249-53.
10. Yesilada E, Sezik E, Honda G, Takaishi Y, Takeda Y, Tanaka T. Traditional medicine in Turkey IX: Folk medicine in north-west Anatolia. J Ethnopharmacol 1999;64:195-210.
11. Poojary R, Kumar NA, Kumarachandra R, Sanjeev G. Evaluation of *in vitro* antioxidant properties of hydro alcoholic extract of entire plant of *Cynodon dactylon*. J Young Pharm 2016;8:378-84.
12. Bharati D, Rawat S, Sharma P, Shrivastava B. Evaluation of *in vivo* efficacy of aqueous extract of aerial parts of *Cynodon dactylon* in rats with simultaneous Type 2 diabetes and hypertension. Curr Bioact Compd 2016;12:25-33.
13. Madhankumar SJ. Protective effect of *Cynodon dactylon* aqueous extract in streptozotocin diabetes induced liver damage in rats-histological study. Int J Pharm Clin Res 2016;8:137-41.
14. Pawaskar SM, Sasangan KC. *In vitro* antimicrobial activity of *Cynodon dactylon* (L.) Pers. leaf extract. Antimicrobial activity by zone of inhibition estimation. Indian Drugs 2015;52:37-41.
15. Kumar SJ, Sundarapandian S, Jebakani CF. Histological and biochemical study on hypoglycemic and anti-hyperlipidemic effects of aqueous extract of *Cynodon dactylon* in streptozotocin-induced diabetic rats. Int J Phytomed 2015;7:23-33.
16. Kumar R, Goyal PK, Mittal A, Pandey A. Evaluation of herbal formulation PACT for wound healing potential. Pharm Lett 2015;7:89-93.
17. Bhangale J, Acharya S. Antiarthritic activity of *Cynodon dactylon* (L.) Pers. Indian J Exp Biol 2014;52:215-22.
18. Paranjpe P. Indian medicinal plants: Forgotten healers. A Guide to Ayurvedic Herbal Medicine with Identity, Habitat, Botany, Photochemistry, Ayurvedic Properties, Formulations and Clinical Usage. 1st ed. Delhi: Chaukhamba Sanskrit Pratishthan; 2001. p. 75-6.
19. Jazani NH, Mikaili P, Shayegh J, Haghighi N, Aghamohammadi N, Zartoshti M. Evaluation of antibacterial activity of *Cynodon dactylon* on multi-drug resistant bacterial isolates in comparing with ciprofloxacin. J Am Sci 2011;7:645-50.
20. Li BJ, Liu Y, Gu AT, Wang F. Chemical constituents of *Cynodon dactylon*. Zhongcaoyao 2017;48:62-6.
21. Abhishek B, Thakur A. Anthelmintic activity of *Cynodon dactylon*. J Pharmacogn Phytochem 2012;1:1-3.
22. Joshi BR, Kommuru DS, Terrill TH, Mosjidis JA, Burke JM, Shakya KP, *et al.* Effect of feeding sericea lespedeza leaf meal in goats experimentally infected with *Haemonchus contortus*. Vet Parasitol 2011;178:192-7.
23. Mechineni A, Kommuru DS, Gujja S, Mosjidis JA, Miller JE, Burke JM, *et al.* Effect of fall-grazed sericea lespedeza (*Lespedeza cuneata*) on gastrointestinal nematode infections of growing goats. Vet Parasitol 2014;204:221-8.
24. Andreassen J. Immunity to adult cestodes: Basic knowledge and vaccination problems. A review. Parassitologia 1991;33:45-53.
25. Yadav AK, Tangpu V. Therapeutic efficacy of *Zanthoxylum rhetsa* DC extract against experimental *Hymenolepis diminuta* (*Cestoda*) infections in rats. J Parasit Dis 2009;33:42-7.
26. Nath P, Yadav AK. Anticestodal properties of *Hibiscus rosa-sinensis* L. (*Malvaceae*): An *in vitro* and *in vivo* study against *Hymenolepis diminuta* (Rudolphi, 1819), a zoonotic tapeworm. J Parasit Dis 2016;40:1261-5.
27. Anonymous. Manual of Veterinary Parasitological Techniques, Technical Bulletin No. 18. London: Ministry of Agriculture, Fisheries and Food, Her Majesty's Stationery Office; 1977. p. 1-57.
28. OECD. OECD Guidelines for the Testing of Chemicals, Acute Oral Toxicity-Up-and-Down-Procedure (UDP), No. 425; 2008. p. 27.
29. OECD. OECD Guidelines for the Testing of Chemicals, Repeated Dose 28-Day Oral Toxicity Study in Rodents, No. 407; 1995. p. 8.
30. Nath P, Yadav AK. Acute and sub-acute oral toxicity assessment of the methanolic extract from leaves of *Hibiscus rosa-sinensis* L. in mice. J Intercult Ethnopharmacol 2015;4:70-3.
31. Mukherjee PK. Evidence-Based Validation of Herbal Medicine. Amsterdam: Elsevier Inc.; 2015. p. 537.
32. Yadav AK, Temjenmongla, Deori K. *In vitro* and *in vivo* anthelmintic effects of *Gynura angulosa* (Compositae) leaf extract on *Hymenolepis diminuta*, a zoonotic tapeworm. J Biol Act Prod Nat 2014;4:171-8.
33. Sujon MA, Mostofa M, Jahan MS, Das AR, Rob S. Studies on medicinal plants against gastroinstestinal nematodes of goats. Bangladesh J Vet Med 2008;2:179-83.
34. Akhtar MS, Ahmad I. Comparative efficacy of *Mallotus philippinensis* fruit (Kamala) or Nilzan drug against gastrointestinal cestodes in Beetal goats. Small Rumin Res 1992;8:121-8.
35. Athanasiadou S, Kyriazakis I. Plant secondary metabolites: Antiparasitic effects and their role in ruminant production systems. Proc Nutr Soc 2004;63:631-9.
36. Ramírez JH, Palacios M, Tamayo O, Jaramillo R, Gutiérrez O. Acute

and subacute toxicity of *Salvia scutellarioides* in mice and rats. J Ethnopharmacol 2007;109:348-53.
37. Tennekoon KH, Jeevathayaparan S, Kurukulasooriya AP, Karunanayake EH. Possible hepatotoxicity of *Nigella sativa* seeds and *Dregea volubilis* leaves. J Ethnopharmacol 1991;31:283-9.
38. Al-Habori M, Al-Aghbari A, Al-Mamary M, Baker M. Toxicological evaluation of *Catha edulis* leaves: A long term feeding experiment in animals. J Ethnopharmacol 2002;83:209-17.

Source of Support: Nil, Conflict of Interest: None declared.