International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 1, 2016

Short Communication

SYNERGISTIC EFFECT OF TWO COMBINATIONS OF *SENNA* PLANT ON THE TEGUMENT OF A RAT TAPEWORM *HYMENOLEPIS DIMINUTA*

SUMAN KUNDU[a], SAPTARSHI ROY[a], LARISHA M. LYNDEM*[a]

[a]Parasitology Research Laboratory, Department of Zoology, Visva-Bharati University, Santiniketan 731235, West Bengal, India
Email: lyndemlarisha@gmail.com

Received: 26 Sep 2015 Revised and Accepted: 02 Dec 2015

ABSTRACT

Objective: Traditional systems of medicine have been adopted in different health care systems worldwide. Amongst them, plant and plant parts have been the most potential based. Our present study was conducted on the treatment of tapeworm *Hymenolepis diminuta* with two combinations of three *Senna* plant, *S. alata* (Sal), *S. alexandrina* (Sax) and *S. occidentalis* (Soc).

Methods: Alcoholic extracts were prepared for individual plant and extracts obtained were mixed in a ratio of 1:1as Sal+Sax, Sal+Soc and Sax+Soc in phosphate buffer saline (PBS) and 1% Dimethyl sulfoxide (DMSO) at pH 7.4 to find out if any synergistic anthelmintic effect occur, since individual plant have already shown vermifugal effect on the parasite in our earlier studies.

Results: Dose-dependent efficacy was observed in all concentrations and in all combinations. Scanning electron micrographs (SEM) showed distortion in the scolex region with shrinkage all over the body tegument; infoldings were observed in proglottids compared to control. Time of paralysis was comparatively less than individual treatment.

Conclusion: Our observation suggests that a combination of two plants is having a synergistic anthelmintic effect on the tapeworm, which could be exploited further in combination drug therapy. Further studies are required to isolate the phytochemical constituents of each *Senna* plant.

Keywords: *Hymenolepis diminuta*, Synergistic, Tegument, Shrinkage.

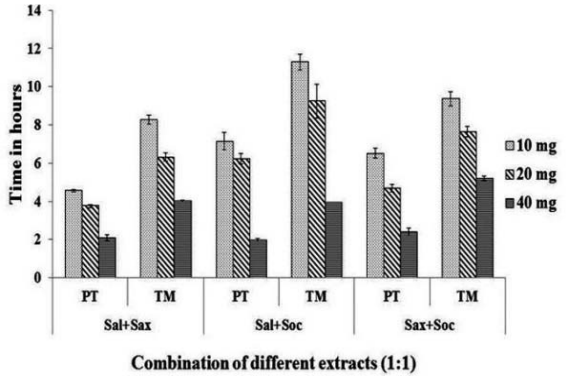
Natural products play a dominant role in the development of novel drug leads for the treatment and prevention of diseases. With the worldwide interest in adopting and studying traditional systems and exploiting their potential based on different health care systems, there is increasing focus on global searches for new drugs derived from natural plant resources. The genus *Senna* (Family: Leguminosae) comprises of 580 species of herbs, shrubs and trees, which are widely distributed throughout the world, of which only twenty species are indigenous to India [1]. Many of the *Senna* spp. possesses a good amount of medicinal properties. Some of which like *Senna alata* (Sal), *S. alexandrina* Vahl (Sax) and *S. occidentalis* Linn (Soc) have been reported in our earlier studies to have anthelmintic effect on flatworms [2-4]. A decoction of the leaves of Sax and Sal was widely practiced in India [5] as compared to other plants. Such type of combination chemotherapy was also common in medical practices in several medical fields such as cancer, bacterial infections, HIV or malaria [6-8] as well as in veterinary medicine [9]. Recent studies made by Klongsiriwet *et al.* (2015) [10] showed a synergistic effect of two plant compounds in inhibition of escheatment of *Haemonchus contortus* (cattle round worm). Thus, it is interesting to explore if the combination of crude extracts of Sal+Sax, Sal+Soc and Sax+Soc are having any synergistic anthelmintic effect on a zoonotic tapeworm *Hymenolepis diminuta.*

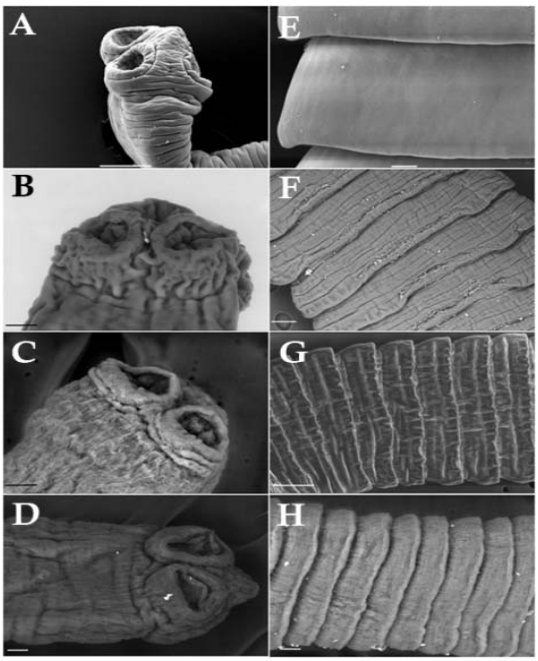
Fresh leaves of the three *Senna* plant were oven dried and crushed to powder. The latter were mixed with ethanol and extracted in Soxhlet apparatus, and the final crude extracts of each leaf were recovered through rotary evaporator. The parasite *H. diminuta* was maintained in our animal laboratory as described in our previous communication [3]. After infection, rats were anesthesized with chloroform and autopsied. Recovered live worms from intestine were exposed to 10, 20 and 40 mg/ml concentration (same set of concentration used for individual plant studies by Kundu *et al.*2012; 2015) of combined alcoholic extracts of Sal+Sax, Sal+Soc and Sax+Soc in the ratio of 1:1 dissolved in PBS with 1 %DMSO. One set of control was simultaneously maintained in PBS with 1% DMSO.

Paralysis of worms was recorded when no movement of any sort could be observed except when shaken vigorously and mortality was recorded when worms showed no movement even when shaken or when dipped in slightly warm PBS. After paralysis, worms exposed to 40 mg/ml concentration were fixed immediately for SEM as described by Roy and Tandon [11].

Amongst the three combinations, paralysis of worms in Sal+Soc occurred at 1.97±0.07 h followed by Sal+Sax (2.09±0.16h) and Sax+Soc (2.4+_0.19h) in 40 mg/ml concentration. The same pattern was revealed in mortality as well, but at 10 mg/ml concentration, worms exposed to Sal+Sax showed early paralysis at 4.56±0.07 h followed by Sax+Soc (6.51±0.25 h) and Sal+Soc (7.14±0.45h), (fig. 1). However, control parasite survived up to 69.22±0.23h. Though in all experimental concentrations there is variation in the time to paralyze the worms, but once it gets paralyzed, it took very short time for the parasites to die. This may be suggested that combination of plant extracts possesses vermifugal activity in nature and may exert a reversible action on the neuromuscular system of the worms and the inactiveness caused would lead the parasite to be swept out of the host's body [12]. Dose-dependent efficacy was also observed with exposure to various concentrations of each combination, as an increase in concentration, shortens the paralysis period. Similar observations were recorded in our earlier studies when *H. diminuta* was treated with single leaf extracts of Sal and Soc respectively [2, 4] as well as reported in many other ethnomedicinal plants [13,14]. SEM studies revealed irreversible damage in the tegumental contour of the whole body, shrinkage and distortions in the scolex and suckers as well as infoldings in the proglottids were observed in our study (fig. 2B,C and D) compared to control (fig. 2A). This may be due to hyperpolarization in the muscle membrane of treated worms and reducing excitability that could lead to muscle relaxation and flaccid paralysis. These changes were also seen when treated with single species of *Senna* [2, 4]. Our present study also revealed vacuolization on the treated worm's surface (fig. 2F,G and H) compared to control (fig. 2E). Such changes are common features observed in other helminths treated with anthelmintic plants [15, 16]. This may be regarded as a stress response resulting from emergency repair to a damaged tegument induced by many harmful elements [17]. However this combination treatment showed more

damaged caused in terms of morphology and the time taken for paralysis were shorten than that of our earlier studies with single plant treatment [2, 4]. This may be suggested that in combination the two plants may have a synergistic effect, and the combined action may have an efficacious intervention on the target organisms. Such observations were also reported by Lyndem *et al.* [18].





CONCLUSION

Thus, these plants besides having vermifugal/vermicidal activity also showed a synergistic effect when treated in combination. However to understand the therapeutic components of activity, isolation of active compounds needs to be investigated.

ACKNOWLEDGEMENT

This work was financially supported by the University Grants Commission (UGC) New Delhi for providing financial assistance through a major research project (No: UGC/SR/40-385/2011) sanctioned to Larisha M. Lyndem and UGC Research Fellowship in Science for Meritorious Students to the first author. We also wish to thank the Department of Zoology, Visva Bharati for providing infrastructural support.

CONFLICT OF INTERESTS

Declared none.

REFERENCES

1. Anonymous. The Wealth of India. Raw Materials. Vol. II. CSIR: Delhi; 1950. p. 93-98.
2. Kundu S, Roy S, Lyndem LM. *Cassia alata* L.: potential role as an anthelmintic agent against *Hymenolepis diminuta*. Parasitol Res 2012;111:1187-92.
3. Kundu S, Roy S, Lyndem LM. Broad spectrum anthelmintic potential of *Cassia* plants. Asian Pac J Trop Biomed 2014;4(Suppl):930-5.
4. Kundu S, Roy S, Nandi S, Ukil B, Lyndem LM. *In vitro a*nthelmintic effects of *Senna occidentalis* (L.) Link (Leguminosae) on rat tapeworm *Hymenolepis diminuta.* Int J Pharm Pharm Sci 2015;7:268-71.
5. Kritikar KR, Basu BC. Indian medicinal plants. Vol II. 2nd edn. Lalit Mohan Basu: Allahabad, India; 1918. p. 870.
6. Keiser J, Utzinger J. The drugs we have and the drugs we need against major helminth infections. Adv Parasitol 2010;73:197–230.
7. White N. Antimalarial drug resistance and combination chemotherapy. Philos Trans R Soc B 1999;354:739–49.
8. Jia J, Zhu F, Ma X, Cao Z, Li Y, Chen YZ. Mechanisms of drug combinations: interaction and network perspectives. Nat Rev Drug Discovery 2009;8:111–28.
9. Bartram DJ, Leathwick DM, Taylor MA, Geurden T, Maeder SJ. The role of combination anthelmintic formulations in the sustainable control of sheep nematodes. Vet Parasitol 2012;186:151–8.
10. Klongsiriwet C, Quijada J, Williams AR, Mueller-Harvey I, Williamson EM, Hoste H. Synergistic inhibition of *Haemonchuscontortus*exsheathment by flavonoid monomers and condensed tannins. Int J Parasitol: Drugs Drug Resist 2015;5:127-34.
11. Roy B, Tandon V. Usefulness of tetramethylsilane in the preparation of helminth parasites for scanning electron microscopy. Riv Parasitol 1991;8:207-15.
12. Martin RJ. Y-aminobutyric acid and Piperazine activated single channel currents from *Ascaris suum* body muscle. Br J Pharmacol 1985;84:445-61.
13. Sujith S, Sreedevi R, Priya MN, Deepa CK, Darsana U, Sreeshitha SG, *et al. the* Anthelmintic activity of three indian medicinal plants. J Pharmacogn Phytochem 2015;7:361-4.
14. Kamal Mostafa ATM, Chowdhury KAA, Hossen Chy MM, Shill LK, Chowdhury S, Hossen Chy MA, *et al.* Evaluation of the anthelmintic activity of seeds of *Sesamumindicum* L. and fruits of *Capsicum frutescens* L. J Pharmacogn Phytochem 2015;3:256-9.
15. Williams AR, Fryganas C, Ramsay A, Harvey IM, Thamsborg SM. Direct anthelmintic effects of condensed tannins from diverse plant sources against *Ascaris suum*. Plos One 2014;9:e97053. doi: 10.1371/journal.pone.0097053. [Article in Press]
16. Williams AR, Ropiak HM, Fryman as C, Desrues O, Harvey IM, Thamsborg SM. Assessment of the anthelmintic activity of medicinal plant extracts and purified condensed tannins against free-living and parasitic stages of *Oesophagos-tomumdentatum*. Parasites Vectors 2014;7:518.
17. Stitt AW, Fair weather I. The effect of the sulphoxide metabolite of triclabendazole (Fasinex) on the tegument of mature and immature stages of the liver fluke, *Fasciola hepatica*. Parasitological 1994;108:555–67.
18. Lyndem LM, Tandon V, Das B. Antihelmintic efficacy of medicinal plants from Northeast India against hookworms: an *in vitro* study on *Ancylostomaceylanium*. Pharmacologyonline 2008;3:697-707.