Available online on www.ijtpr.com
International Journal of Toxicological and Pharmacological Research 2015; 7(3); 130-134

ISSN: 0975-5160

Research Article

In-vitro Intestinal Activity of Leaves Extract of *Dryopteris chrysocoma*

Noor-Jahan[1*], Mansoor Ahmad[2], Mehjabeen[3], Farah-Saeed[4], Shafi Muhammad[5], Asif Bin Rehman[6]

[1] *Department of Pharmacology, Dow College of Pharmacy, Dow University of Health Sciences, Karachi-74200, Pakistan.*
[2] *Department of Pharmacognosy, Research Institute of Pharmaceutical Sciences, University of Karachi, Karachi-75270, Pakistan.*
[3] *Department of Pharmacology, Faculty of Pharmacy, Federal Urdu University for Science, Arts & Technology, Karachi, Pakistan.*
[4] *Department of Pharmacognosy, Dow College of Pharmacy, Dow University of Health Sciences, Karachi-74200, Pakistan.*
[5] *Department of Pharmacognosy, Faculty of Pharmacy, University of Baluchistan, Quetta, Pakistan*
[6] *Department of Pharmacology, Hamdard University, Karachi, Pakistan.*

Available Online: 27th April, 2015

ABSTRACT

The male Filix-mas is one of the evergreen fern in different regions of Pakistan. It is abundantly found in moist and damp areas. The rootstock of the male fern has medicinal properties. The active principles are phloroglucinol derivatives. Filmarone and aspidinol are its constituents. The root extract causes laxation, it is also wormicidal but it has hepatotoxicity. The crude extract of leaves of *D. chrysocoma* was compared with standards as acetylcholine, atropine, adrenaline, neostigmine, metoclopramide and pheniramine. *In-vitro* effect of *D. chrysocoma* leaves extract was determined on isolated rabbit jejunum post treated with acetylcholine $1x10^{-4}$M, adrenaline $1x10^{-2}$M, pheniramine $1x10^{-2}$M and metoclopramide $5x10^{-2}$M. Crude extract of *D. chrysocoma* (leaves) showed smooth muscle relaxant effect. The leaf extract of *D. chrysocoma* showed slight muscle relaxant activity. The muscle relaxation increased as the dose increased. After relaxation the tissue comes to its normal condition, slight wavy pattern of movement was observed. Intestinal movement becomes regular and normal before washing while after washing intestinal motility increases. The crude extract of *D. chrysocoma* (leaves) produced its relaxant effect by adrenergic receptors. The drug potentiated the action of acetylcholine so the drug can be used to improve gastrointestinal activity.

Key words: *D. chrysocoma*, male fern, in-vitro intestinal activity, smooth muscle relaxant effect

INTRODUCTION

D. chrysocoma is found in most areas of Asia. It is found in warm and dry shady areas. The plant is also called male fern. It is evergreen. The height is not more than one and half meters. Roots and rhizomes are commonly used for certain ailments. The plant contains oleoresin, filicic acid, flavaspidic acid, filmaron, albaspidin[1]. The rhizome contain phloroglucinol derivatives of crude filicin consisted of albaspidin, filixic and flavaspidic acids[2]. Haas *et al.* in 1991 tested the alkaloid staurosporine, currently known as the most potent inhibitor of protein kinase C, PKC, for its ability to inhibit phytochrome-mediated spore germination in *D. filix-mas* L[3]. The plant was eaten raw as part of a regime for losing weight[4].

Externally, the root is used as a poultice in the treatment of abscesses, boils, carbuncles and sores[5,6]. Fraunfelder in 2004 reported the use of *D. filix mas* in ophthalmic disorders[7]. The malarial fever is cured by leaf extract of *Ajuga bracteosa*, *Dryopteris filixmas* and also at times by *Picrorhiza kurrooa*[8]. The root contains an oleoresin that paralyses tapeworms and other internal parasites and has been used as a worm expellant[9]. The active taenicidal ingredients are derivatives of phenolic compounds phloroglucinol. Roots contain filicin that is active against tape worms. Tape worms expelled by the use of 1 to 2 drams of tincture on an empty stomach in the morning. This was to be followed with a laxative that evening and the following morning if necessary. Patient should fast on pineapple, flax seeds and pumpkin seeds for two days making sure to chew the seeds and nuts thoroughly and drinking plenty of water[10]. Blakemore *et al.* in 1964 tested various extracts, oils, and individual phloroglucinol (I) compounds prepared from the fern *Dryopteris dilatata* and *D. filixmas* for taeniacidal activity against the dwarf tapeworm. It is effective against tape worms in high doses but high doses can damage liver[11]. It is abortifacient so pregnant women and people with heart complaints should not be prescribed this plant[12].

MATERIALS AND METHODS

Preparation of Plant Material

Fresh leaves of *D. chrysocoma* were collected from

**Author for Correspondence*

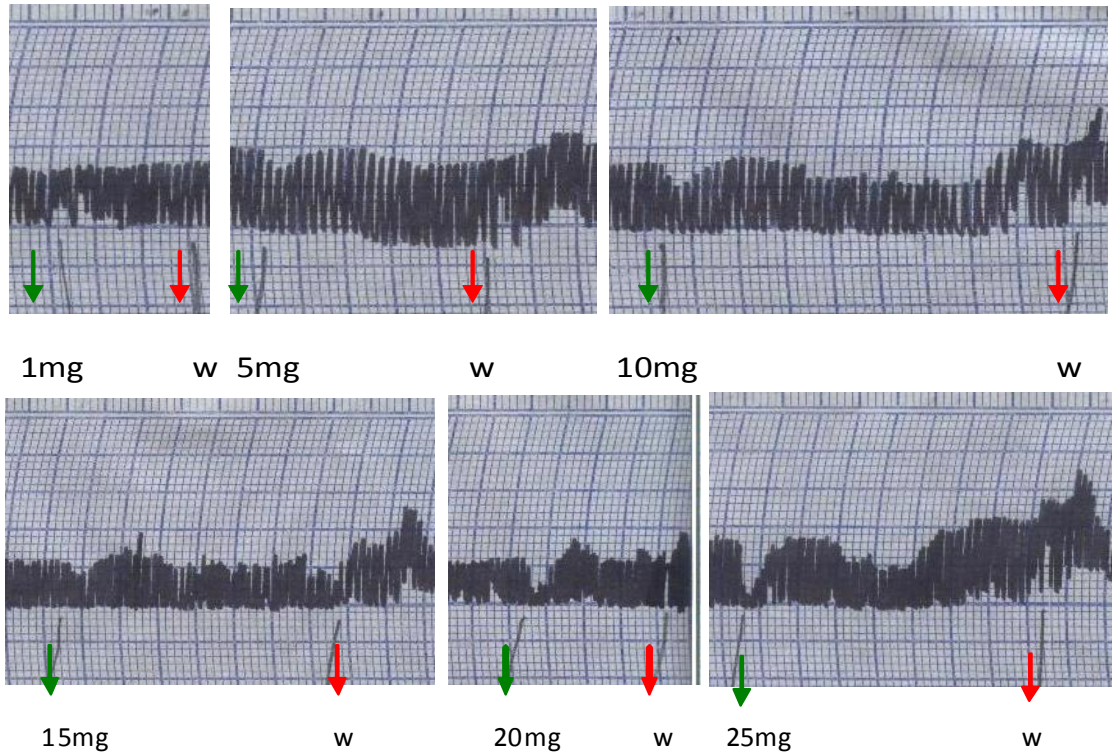


Figure 1: Tracings showing *in vitro* smooth muscles activity of *D. chrysocoma* (Leaves) on rabbit Jejunum

Botanical garden of University of Karachi. A specimen of *Dryopteris chrysocoma* (leaves) was submitted in herbarium (herbarium number, 001006-08). The leaves were washed; air dried and cut in small pieces. Then it was soaked in methanol at room temperature for 15 days. After that the extract was filtered using filter paper and dried on Rotary evaporator, Eyela (Japan). A thick residue obtained that was used as drug for experiment.

Animals

Swiss albino rabbits of either sex of 1 kg weight were used for experiment.

Smooth Muscles Activity

Smooth Muscles Preparation

Rabbit was sacrificed by a blow on the back neck. Then intestines was removed immediately from abdomen and placed in Tyrode's solution in beaker with continuous supply of oxygen. Small piece of jejunum or ileum of about 3cm was cut and hang with the help of thread in organ bath having 70ml capacity that was filled with Tyrode's solution. In the water bath circulating temperature was maintained at 37℃ and perfuse with a mixture of carbogen[13].

Assay Method

The intestine segment was allowed to equilibrate before starting the experiments. The spontaneous movements of intestine were recorded on Oscillo-graph or polygraph using isotonic transducer[13].

To determine the effects of plant extract on spontaneous movements of intestine, 0.1 g of crude dry extract was dissolved in 2 or 3mL of distilled water and thereafter, it was added to the organ bath after equilibration period.

The effects of crude extracts on the contraction and relaxation pattern of isolated rabbit intestine (smooth muscles) are recorded in Tables.

RESULTS

Crude extract of *D. chrysocoma* (leaves) showed smooth muscle relaxant effect (table 1, figure 1, and graph 1). The leaf extract of *D. chrysocoma* showed slight muscle relaxant activity. The muscle relaxation increased as the dose increased. The crude extract was observed in different concentrations that is 1 to 25mg/ml. The effects were observed by direct administration on isolated intestinal segment. Intestinal motility was increased after washing that indicates that it is not toxic for the isolated segment. At 1mg dose 12.5% response was obtained with t-value 1.224. The response increased with the increase in dose and at 15 mg it was 50% at p value 0.01. The result was also significant at 20 mg that was 60% (p=0.03). The highest dose of crude extract on which the observations were observed was 25mg/ml. Highly significant response was observed at this dose that was 75% (p=0.008). The results were compared using standard drugs in different concentrations as drugs acting on muscarinic, adrenergic, dopamine or histamine receptors. The isolated intestinal segment was pre and post treated with acetylcholine and it was found that it increased the response of acetylcholine. When the intestinal segment was pre and post treated with adrenaline than full response of adrenaline that is inhibition of intestinal activity was not observed that indicated that adrenergic receptors were involved there. When the isolated segment was pre and post treated with drug that is leave extract with the standards acting on dopamine that is avil and metoclopramide that act on dopamine receptors, no change an activity was observed. The crude extract of *D. chrysocoma* (leaves) produced its relaxant effect by adrenergic receptors. The drug

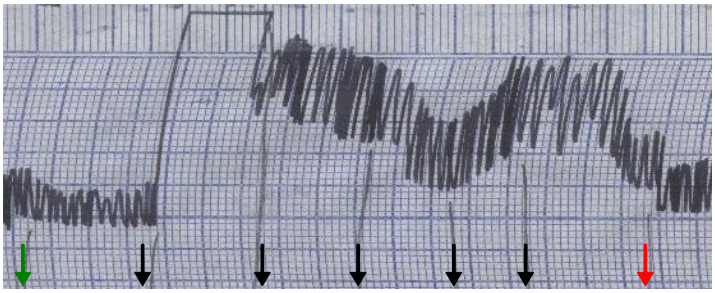


Figure 2a: Tracing showing *in vitro* effect of *D. chrysocoma* leaves extract on isolated rabbit jejunum post treated with acetylcholine $1x10^{-4}$M, adrenaline $1x10^{-2}$M, avil $1x10^{-2}$M and metoclopramide $5x10^{-2}$M

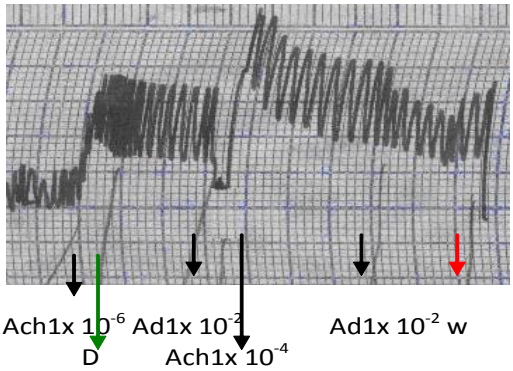


Figure 2b: Effect of leaves extract on tissue pre treated with acetylcholine $1x10^{-6}$M and post treated with adrenaline $1x10^{-2}$M and acetylcholine $1x10^{-4}$M

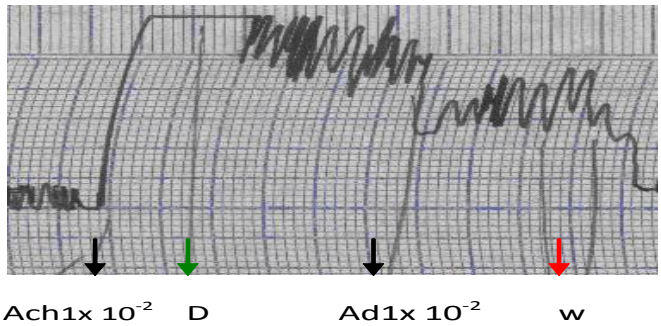


Figure 2c: Tracing showing effect of leaves extract on isolated jejunum pretreated with acetylcholine $1x10^{-2}$M and post treated with adrenaline $1x10^{-2}$M

Table 1: Dose related response of crude extract of *D. chrysocoma* (Leaves) on isolated rabbit intestine

Dose (mg/ml)	Control (cm)	Response (cm)	Response in Percentage	t- value	p-value
01	0.8±0.057	0.7± 0.057	12.5	1.224	0.143
05	1±0.2	0.8± 0.2	20	1.224	0.143
10	0.8±0.115	0.5 ± 0.10	37.5	1.963	0.060
15	0.6±0.057	0.3± 0.057	50	3.674*	0.010
20	0.5±0.10	0.2± 0.057	60	2.598*	0.030
25	0.8±0.115	0.2± 0.088	75	3.899**	0.008

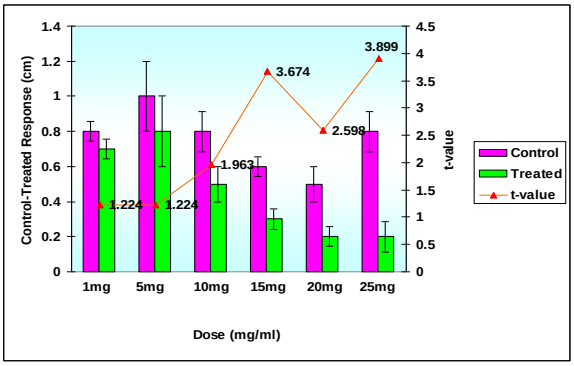
The results are expressed in at $P \leq 0.05$ and $P \geq 0.05$; *significant, **highly significant

potentiated the action of acetylcholine (figure 2a-c, graph 2a-c).

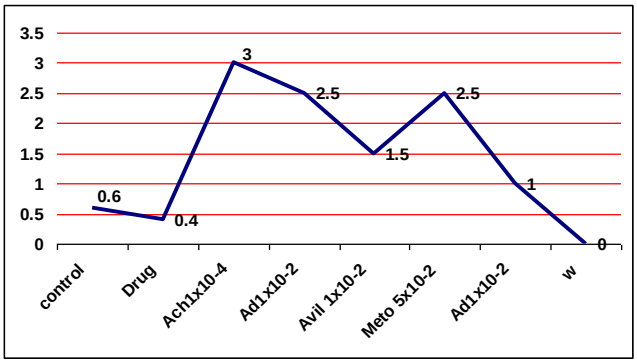
DISCUSSION

In vitro smooth muscle activity on rabbit intestine showed in table 1 with their tracings (figure 1). Crude extract of *D. chrysocoma* (leaves) showed smooth muscle relaxant effect. The leaf extract of *D. chrysocoma* show slight muscle relaxant activity. The muscle relaxation increased as the dose increase. After relaxation the tissue comes to its normal condition, slight wavy pattern of movement was observed. Intestinal movement becomes regular and normal before washing while after washing intestinal motility increases (graph 1).

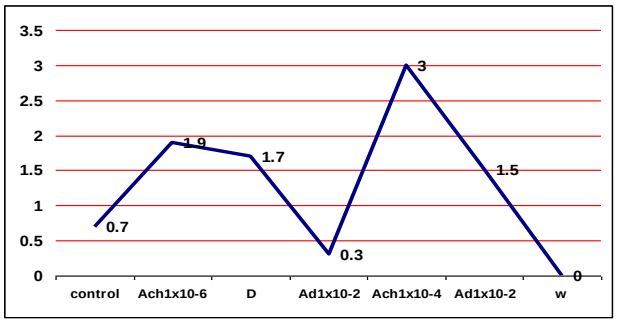
Each experiment on smooth muscles was carried out at least three times. The crude extract was compared with acetylcholine, atropine, adrenaline, neostigmine, metoclopramide and avil (figure 2a, b and c). Saito *et al.* in 1996 claimed flavon-3-ol structure-containing glycosides as histidine decarboxlase inhibitors and compounds containing the inhibitors for treatment of e.g. peptic ulcers and atopic dermatitis. The flavon-3-ol structure-containing glycosides were obtained by enzymic synthesis or by extraction from medicinal plants such as *D. filix-mas* and phyllocladus trichomanoides[14]. Akhtar *et al.* in 1990 worked on L-methionine decarboxylase: kinetics and mechanism of decarboxylation and abortive transamination. L-Methionine decarboxylase from *D. filix-mas* catalyzes the decarboxylation of L-methionine and a range of straight- and branched-chain L-amino acids to give the corresponding amine products[15]. In present study figure 2a showing tracing of *in-vitro* effect of *D. chrysocoma* leaves extract on isolated rabbit jejunum post treated with acetylcholine $1x10^{-4}$M, adrenaline $1x10^{-2}$M, avil $1x10^{-2}$M and metoclopramide $5x10^{-2}$M. When a tissue treated with drug was post treated with acetylcholine ($1x10^{-4}$ M) it produced its full effect showing that the drug



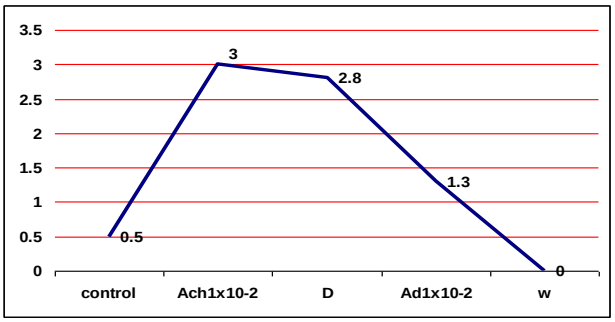
Graph 1: Dose related response of crude extract of *D. chrysocoma* (Leaves) on isolated rabbit intestine



Graph 2a: Tracing showing *In vitro* effect of leaves extract on isolated jejunum post treated with acetylcholine $1x10^{-4}$M, adrenaline $1x0^{-2}$M, avil ($1x10^{-2}$M and metoclopramide $5x10^{-2}$M)



Graph 2b: Effect of leaves extract on tissue pre treated with acetylcholine $1x10^{-6}$M and post treated with adrenaline $1x10^{-2}$M and acetylcholine $1x10^{-4}$M



Graph 2c: Effect of leaves extract on isolated jejunum pretreated with acetylcholine $1x10^{-2}$M and post treated with adrenaline $1x10^{-2}$M

potentiates the action of acetylcholine. When adrenaline ($1x10^{-2}$ M) administered it did not produced its effects as adrenergic receptors were blocked, the administration of avil ($1x10^{-2}$M) produced its full effect similarly metoclopramide ($5x10^{-2}$M) produced its full effect showing no involvement of histamine and dopamine receptors. Post treatment with adrenaline ($1x10^{-2}$ M) again did not produce its effect showing adrenergic receptor blockage (graph 2a). Figure 2b showing effect of leaves extract on tissue pre treated with acetylcholine $1x10^{-6}$M and post treated with adrenaline $1x10^{-2}$M and acetylcholine $1x10^{-4}$M. When a pretreated tissue with acetylcholine ($1x10^{-6}$ M) was treated with drug it produced its effect. When the tissue was treated with adrenaline ($1x10^{-2}$ M) it did not produced its full effect due to blockage of adrenergic receptors. The administration of acetylcholine ($1x10^{-4}$ M) produced its full effect showing that muscarinic receptors were not involved. Figure 2c showing that when a pretreated tissue with acetylcholine ($1x10^{-2}$ M) was treated with drug it did not produced its effect while post treatment with adrenaline ($1x10^{-2}$ M) produced its slight effect as the adrenergic receptors were blocked (graph 2c). The crude extract of *D. chrysocoma* (leaves) produces its relaxant effect by adrenergic receptors. The drug potentiates the action of acetylcholine.

CONFLICT OF INTEREST

Necessary Permissions were taken from statuary bodies applicable. Authors declare no conflict of Interest.

REFERENCES

1. Bhattacharjee, Kumar S. (2004). Hand Book of Medicinal Plants. 4th revised and enlarged edition. Pointer Publishers, Jaipur 302003 India. p. 138, 348.
2. Asolkar L.V., Kakkar K.K., Chakre O.J. (1992). Second supplemented to Glossary of Indian Medicinal Plants with active principles. Part-I (A-K), 1965-1981. National Institute of Science Communication (CSIR). Dr. K.S. Krishnan Marg. New Delhi-110012. p. 283.
3. Haas C.J., Scheuerlein R., Roux S.J., Roux S.J. (1991). Phytochrome-mediated germination and early development in spores of Dryopteris filix-mas L.: phase-specific and non phase-specific inhibition by staurosporine. J Plant Physiol. 138(6):747-51.
4. Moerman D. (1998). Native American Ethnobotany. Timber Press. Oregon.
5. Bown D. (1995). Encyclopaedia of Herbs and their Uses. Dorling Kindersley, London.
6. Stuart M. (1979). The Encyclopedia of Herbs and Herbalism Orbis Publishing. London.
7. Fraunfelder F.W. (2004). Perspective. Ocular Side Effects From Herbal Medicines and Nutritional Supplements. American J. Ophthalmology 138:639–647.
8. Pushpangadan P., Ulf Nyman, George V. (1995). Glimpses of Indian Ethnopharmacology. Tropical Botanic Garden and research Institute. Thiruvananthapuram 695562, Kerala India, Visual security printing enterprises Pvt. Ltd., New Delhi, p.194.

9. Foster S. & Duke J.A. (1990). A Field Guide to Medicinal Plants. Eastern and Central N. America. Houghton Mifflin Co., ISBN 0395467225.
10. Mitchell, William A. (2003). Plant Medicines in Practice, using the teachings of John Bastyr. Churchill Livingstone, an Imprint of Elsevier Science, p.31, 208, 354.
11. Blakemore R.C., Bowden K., Broadbent J.L., Drysdale A.C. (1964). Anthelmintic constituents of ferns. Journal of Pharmacy and Pharmacology 16(7):464-71.
12. Chiej R. (1984). Encyclopaedia of Medicinal Plants. MacDonald, USA.
13. Staff of the pharmacology department. (1970). University of Edinburgh. Pharmacological experiments on isolated preparations. Chrchill Livingstone. London, p.58-64.
14. Saito M., Kitao S., Ichikawa A. (1996). Flavon-3-ol structure-containing glycosides as histidine decarboxlase inhibitors and pharmaceutical compositions containing the inhibitors. (Noda Sangyo Kagaku Kenkyusho, Japan; Kikkoman Corp). Jpn. Kokai Tokkyo Koho, Patent No. JP 8217674., p.22.
15. Akhtar M., Stevenson D.E., Gani D. (1990). Fern L-methionine decarboxylase: kinetics and mechanism of decarboxylation and abortive transamination. Biochemistry 29(33):7648-60.