

EFFECTS OF BENZODIAZEPINE AND IVERMECTIN ON *Girardia tigrina* (Platyhelminthes: Turbellaria)

EFEITOS DE BENZODIAZEPÍNICO E IVERMECTINA EM *Girardia tigrina* (Platyhelminthes: Turbellaria)

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ABSTRACT: Four hundred specimens of *Girardia (=Dugesia) tigrina* separated in groups of 5 were subjected to concentrations of 10, 25 and 50 ppm diazepam (DZP) and 1, 5 and 10 ppm of ivermectin (IVM), substances with actions associated with ionophore chlorine and GABA receptors in neuromuscular endings in various animals. The period of turbellarians exposure at different concentrations used was 3 h, for DZP while those to IVM were 30, 60, 120 minutes, 12 and 24 h for each tested concentration. In groups exposed to DZP hyperkinetic and type "C" and screw like movements were observed in all concentrations. However, at concentrations of 50 and 25 ppm, the pharynx was protruded in 100% of the specimens followed by detachment at concentration of 50 ppm and 60% at a concentration of 25 ppm. Mortality reached 100% 24 hours after exposure to a concentration of 50 ppm and 30% of planarians became degenerated at a concentration of 25 ppm. Planarians submitted to concentrations of 10 and 25 ppm remained alive and without hyperkinetic movements 48 hours after exposure. In IVM exposed groups, hyperkinetic and contractile movements were observed as well as type "C" screw like movements in all tested concentrations and time dependent. The group exposed for 24 hours showed a time dependent variation in mortality from 0 to 100%. The turbellarians were alive for 48 hours in 1 ppm during 12 hours of exposure but with 24 hours of exposure the mortality reaches 20%. The data indicate a time and concentration dependent relationship in the mode of action of these drugs.

KEYWORDS: Planarian. Ivermectin. Diazepam. Dopamine Receptors. Drug effects.

INTRODUCTION

Planarians also known as free-living platyhelminthes are generally regarded primitive representatives of the metazoan, presenting bilateral symmetry, encephalization and a segmented nervous system. Equally in higher animals, planarians have neurotransmitters in their central nervous system including acetylcholine, norepinephrine, dopamine and serotonin (BULLOCK; NACHMANSON, 1942; WELSH, 1946; LENTZ, 1968; BEST; NOEL, 1969; WELSH, 1972; SUPLAVILAI; KAROBATH, 1980; VENTURINI et al., 1989; HORVAT et al., 2005; PRÁ et al., 2005).

In addition to the classic studies on ecology, physiology and regeneration, these platyhelminthes are being used for neuropharmacological (MORITA; BEST, 1965) and behavioral research with drugs that interfere with dopaminergic-1-receptors (D₁). When these receptors are stimulated there is an increase in adenylate cyclase activity and D₂ dopamine receptors, which when stimulated decreases or has no effect on the production of AMPc (BATTAGLIA et al., 1985; GODDMAN; GILMAN, 2000; SNYDER et al., 2000; BEAULIEU et al., 2006; BUTTARELLI, et al., 2008). Moreover, the use of non-selective

dopaminergic agonists in planarians induces a typical hyperkinetic pattern (screw like hyperkinesia) and a significant increase in the levels of AMPc (WELSH, 1972), whose effects are inhibited by non-selective dopaminergic blocking agents (WELSH; KING, 1970).

There has been an increasing number of studies aiming at assessing the acute effects of toxic substances in populations of aquatic invertebrates (FREITAS et al., 1996; ALVES et al., 2004; 2010), since it is possible to contaminate watercourses by chemicals used in agriculture and animal husbandry (HORVAT et al., 2005; PRÁ et al., 2005) and the effects caused by its indiscriminate use are still inaccurate.

One of these substances, Ivermectin, which is produced from a microorganism, *Streptomyces avermitilis*, is a semisynthetic derivative of avermectins with low water solubility (CAMPBELL et al., 1983; FISHER; MROZIK, 1989), and has been used as an antiparasitic agent in animal treatment against intestinal worms, mites and insects (AZIZ et al., 1982; CAMPBELL et al., 1983; CAMPBELL, 1985).

Avermectins increase the muscle-relaxing effects of diazepam, *in vivo*, and stimulate the binding of [³H]-diazepam on rodent brain

membranes *in vitro* (WELSH; KING, 1970; WELSH; WILLIAMS, 1970; WILLIAMS; YARBROUGH, 1979; SUPLAVILAI; KAROBATH, 1980; WILLIAMS; RISLEY, 1982; NOEL et al., 2007). These observations suggest that avermectins interfere in the GABA-benzodiazepine receptor complex (FISHER; MROZIK, 1989). In fact, some studies show the action of avermectins in neuromuscular endings involving chloride channels and GABA in several species of helminthes and arthropods (LENTZ, 1968; CAMPBELL et al., 1983; ALBERT et al., 1986).

Preliminary observations made in our laboratories reported loss of mobility and mortality with an increasing concentration of Ivermectin on *Biomphalaria glabrata*, probably due to membrane depolarization and problems with the chlorine pump (unpublished data). A similar effect was observed in the third and fourth instar of *Culex quinquefasciatus* exposed to Ivermectin (FISHER; MROZIK, 1989; FREITAS et al., 1996; ALVES et al., 2004; 2010).

The objective of this study was to observe the effects of diazepam (DZP) and Ivermectin (IVM) on *Girardia tigrina* in laboratory conditions.

MATERIAL AND METHODS

Specimens of *Girardia* (= *Dugesia*) *tigrina* from the semi-natural breeding tanks of the Laboratory of Invertebrate Biology and Taxonomy, of the Department of Parasitology were transferred to a petri dish containing tap water and allowed to acclimate at room temperature for 24 h before the experiments were performed.

The turbellarians were separated into groups of 5 specimens for each assay and transferred to disposable plastic containers with solutions of Ivermectin (Ivomec[®] - Merial, Campinas, SP, Brazil) or diazepam (Diempax[□] - Sanofi Aventis, São Paulo, SP, Brazil) prepared on the day of the experiment. *G. tigrina* groups exposed to the IVM solution were submitted to concentrations of 1, 5 and 10 ppm for periods of 30, 60 and 120 minutes, 12 and 24 hours (75 specimens); and those exposed to the DZP solution were submitted to concentrations of 10, 25 and 50 ppm for a period of 3 hours (15 specimens).

Following the exposure periods, the planarians were washed and transferred to different containers with chlorine-free water and were observed with a stereomicroscope immediately, 24 and 48 hours after. The platyhelminthes used as control were kept under the same conditions as the experimental group during the experiment and were

not reused. Cephalic degeneration was considered when the anterior region of Turbellarian became altered and losing fragments. The “type C” and “screw like” movements were analyzed according to Venturini et al. (1989).

Statistical comparison of the data of death and increase of movements of the flatworms between control and test groups at different times and concentrations were performed using a Fisher's exact test for proportions. The level significance of 5% was considered statistically significant.

RESULTS

The results are summarized in Tables 1 and 2.

It was verified that planarians from the control groups (maintained only in tap water) did not display altered movements or hyperkinesias. In contrast, all tested groups exposed to IVM, presented hyperkinesias (“screw like” and “type C” movements) during the period of exposure, and after being washed with dechlorinated water (data not shown).

In addition, with increasing drug concentrations and time, some specimens showed cephalic degeneration and mortality (20 to 100%) in 5 and 10 ppm exposed group for 12 hours and in all tested groups exposed for 24 hours (also 20 to 100%). The groups exposed to DZP, apart from relaxation and “type C” movements, presented, at higher doses (50 ppm), the “screw like” movements between 15 and 150 minutes after the drug exposition and before the start or records (3 and 24h; results not shown).

Comparisons of the mortality rate and disorder of planarians, between the groups and in different periods, were performed in order to verify the significance between the obtained values. The mortality rate of these flatworms, when compared with the control groups, was statistically significant ($p < 0.001$).

Table 1: Number (%) of *G. tigrina* specimens presenting hyperkinesia (Hy) and mortality (M) 24 and 48 hours after different periods of exposure and concentrations of the Ivermectin solution

Exposure Time	Control		Doses						Observations (hours)
			1ppm		5ppm		10ppm		
	Hy (%)	M (%)	Hy (%)	M (%)	Hy (%)	M (%)	Hy (%)	M (%)	
30 minutes	0 (0)	0 (0)	0 (0)	0 (0)	20 (100)*	0 (0)	20 (100)*	0 (0)	24
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	48
60 minutes	0 (0)	0 (0)	0 (0)	0 (0)	20 (100)*	0 (0)	20 (100)*	0 (0)	24
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	48
120 minutes	0 (0)	0 (0)	0 (0)	0 (0)	20 (100)*	0 (0)	20 (100)*	0 (0)	24
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	48
12 hours	0 (0)	0 (0)	20 (100)*	0 (0)	20 (100)*	4 (20)*	20 (100)*	14 (70)*	24
	0 (0)	0 (0)	20 (100)*	0 (0)	20 (100)*	4 (20)*	20 (100)*	20 (100)*	48
24 hours	0 (0)	0 (0)	20 (100)*	4 (20)*	20 (100)*	15 (75)*	20 (100)*	20 (100)*	24
	0 (0)	0 (0)	20(100)*	4 (20)*	20 (100)*	20 (100)*	20 (100)*	20 (100)*	48

= $p < 0.001$ (related to control group)

Table 2: Number (%) of *G. tigrina* specimens presenting disorders after 3 hours exposure to different concentrations of diazepam

Disorders	Control	Doses			Observations (hours)
		10ppm	25ppm	50ppm	
Mortality (%)	0 (0)	0 (0)	0 (0)	0 (0)	3
	0 (0)	0 (0)	0 (0)	20 (100)*	24
	0 (0)	0 (0)	0 (0)	-	48
Pharynx protrusion (%)	0 (0)	20 (100)*	20 (100)*	20 (100)*	3
	0 (0)	0 (0)	0 (0)	-	24
	0 (0)	0 (0)	0 (0)	-	48
Subsequent loss of pharynx (%)	0 (0)	0 (0)	12 (60)*	18 (90)*	3
	0 (0)	0 (0)	12 (60)*	-	24
	0 (0)	0 (0)	12 (60)*	-	48
"C" Movement (%)	0 (0)	20 (100)*	20 (100)*	20 (100)*	3
	0 (0)	0 (0)	0 (0)	-	24
	0 (0)	0 (0)	0 (0)	-	48
Degeneration (%)	0 (0)	0 (0)	3 (15)*	20 (100)*	3
	0 (0)	0 (0)	6 (30)*	-	24
	0 (0)	0 (0)	6 (30)*	-	48

* = $p < 0.0005$ (related to control group)

DISCUSSION

The presence of dopamine in planarians was initially confirmed through chromatography (WELSH; KING, 1970). Subsequently, Venturini et al. (1989) showed that various drugs that affect the receptors of dopamine alter the motor activity in *Girardia*.

The results obtained from this study suggest that D₁/D₂ mixed activity in IVM treated planarians nervous system, since "type C" (typical D₂ pattern) and "screw like" (D₁) movements that occurred in an abrupt manner and increased in numbers of asynchronous movements which are different from the normal locomotor activity observed in control group, are similar in response to the stress induced

by haloperidol, apomorphine and 3,4-dihydroxyphenylalanine (L-DOPA) (CAROLEI et al., 1975; ALGERI et al., 1983; VENTURINI et al., 1989), as well as a possible influence in dopaminergic receptors (PASSARELLI et al., 1999; BUTTARELLI et al., 2000).

Actually, specimens of *Dugesia gonocephala* respond to specific D₂ agonists with a "type C" and a standard hyperkinetic "screw like" pattern, both also observed in treatment with selective D₁ agonists due to an increased endogenous dopamine release (CAROLEI et al., 1975; STEFANO; HIRIPI, 1979; ALGERI et al., 1983).

The current consensus is that most, if not all benzodiazepine actions, result from the potentiation

of neural inhibition mediated by GABA (GODDMAN; GILMAN, 2000). This idea is supported by behavioral and electrophysiological evidence that the effects of BDZ are usually reduced or prevented by previous treatment with GABA antagonists or transmitter synthesis inhibitors. Although it is not possible to exclude potential actions that lead to an increased release of GABA, more attention should be focused on the ability of benzodiazepine to potentiate the actions of GABA on neurons at all levels of the nervous axis. As a result of the detection and characterization of specific binding sites for benzodiazepines, there is a substantial amount of biochemical evidence suggesting a close molecular association between the sites of action of GABA and benzodiazepines

(GODDMAN; GILMAN, 2000; NISHIMURA et al., 2007; 2008; BUTARELLI, et al., 2008).

Moreover, other types of disorders observed during the experiment were the hyperkinesias, which prevailed until the death of planarians when exposed to a higher dose of IVM. In flatworms exposed to larger doses of DZP, body relaxation followed by death was also verified. These facts suggest that during the period of exposure, the drugs might also act on neuromuscular ends with the involvement of chloride channels and GABA, inducing membrane depolarization because *G. tigrina* contains numerous GABA fibers in the central and peripheral nervous system (EGERTON et al., 1979; ERIKSSON; PANULA, 1994).

RESUMO: Quatrocentos exemplares de *Girardia (=Dugesia) tigrina* separados em grupos de 5 foram submetidos a concentrações de 10, 25 e 50ppm de diazepam (DZP) e 1, 5 e 10ppm de ivermectina (IVM), substâncias com ações associadas ao ionóforo cloro e receptores GABA em terminações neuromusculares em vários animais. O tempo de exposição dos turbelários às diferentes concentrações utilizadas de DZP foi de 3 horas, enquanto para cada concentração de IVM os exemplares permaneceram expostos durante 30, 60, 120 minutos, 12 e 24 horas. Nos grupos expostos ao DZP foram verificados movimentos hipercinéticos tipo parafuso e tipo “C” e exposição da faringe ao exterior em todas as concentrações. Houve perda da faringe em 90% dos espécimes na concentração de 50ppm e 60% em 25ppm. Foi verificado também 100% de mortalidade após 24 horas em concentração de 50ppm e 30% de degeneração dos platyhelminthes na concentração de 25ppm. No exame realizado 48 horas após a exposição, as planárias submetidas às concentrações de 25 e 10 ppm permaneceram vivas e não mais apresentaram movimentos hipercinéticos. Nos grupos expostos à IVM, foram observados movimentos contráteis e movimentos hipercinéticos tipo parafuso e tipo “C” em todas as concentrações e foi dependente do tempo de exposição. O percentual de mortalidade variou de 0 a 100 nas primeiras 24 horas de observação também dependente do tempo de exposição. Em concentração de 1 ppm e até 12 horas de exposição não foi observada mortalidade dos animais até 48 horas após. Entretanto com 24 horas de exposição e exame 24 horas após a mortalidade chega a 20% dos exemplares persistindo até o final do período de observação. Os dados indicam uma relação dependente da concentração e do tempo no modo de ação destas substâncias.

PALAVRAS-CHAVE: Planária. Ivermectina. Diazepam. Receptores dopaminérgicos. Efeito de drogas.

REFERENCES

- ALBERT, J.; LINGLE, D. H.; MARDER, E.; O'NEIL, M. B. A GABA-activated chloride-conductance not blocked by picrotoxin on spiny lobster neuromuscular preparations. **British Journal of Pharmacology**, v. 87, n. 4, p. 771-779, apr. 1986.
- ALGERI, S.; CAROLEI, A.; FERRETTI, P.; GALLONE, C.; PALLADINI, G.; VENTURINI, G. Effects of dopaminergic agents on monoamine levels and motor behaviour in planaria. **Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology**, v. 74, n. 1, p. 27-29, jan. 1983.
- ALVES, S. N.; SERRÃO, J. E.; MELO, A. L. Alterations in the fat body and midgut of *Culex quinquefasciatus* larvae following exposure to different insecticides. **Micron**, v. 41, n. 6, p. 592-597, aug. 2010.
- ALVES, S. N.; SERRÃO, J. E.; MOCELIN, G.; MELO, A. L. Ivermectin effects on the life cycle and larval fat body of *Culex quinquefasciatus* (Say, 1823) (Diptera, Culicidae). **Brazilian Archives of Biology and Technology**, v. 47, n. 3, p. 433-499, jul. 2004.

AZIZ, M. A.; DIOP, I. M.; DIALLO, S.; LARIVIERE, M.; PORTA, M. Efficacy and tolerance of ivermectin in human Onchocerciasis. **The Lancet**, v. 320, n. 8291, p. 171-173, jul. 1982.

BATTAGLIA, G.; NORMAN, A. B.; HESS, E. J.; CREESE, I. D2dopamine receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in rat striatum. **Neuroscience Letter**, v. 59, n. 2, p. 177-182, aug. 1985.

BEAULIEU, J. M.; SOTNIKOVA, T. D.; GAINETDINOV, R. R.; CARON, M. G. Paradoxical Striatal Cellular Signaling Responses to Psychostimulants in Hyperactive Mice. **The Journal of Biology Chemistry**, v. 281, n. 43, p. 32072-32080, oct. 2006.

BEST, J. B.; NOEL, J. Complex synaptic configurations in planarian brain. **Science**, v. 164, n. 3883, p. 1070-1071, may 1969.

BULLOCK, T. H.; NACHMANSON, D. Choline esterase in primitive nervous system. **Journal of Cellular and Comparative Physiology**, v. 20, n. 2, p. 239-242, oct. 1942.

BUTTARELLI, F. R.; PONTIERI, F. E.; MARGOTTA, V.; PALLADINI, G. Acetylcholine/dopamine interaction in planaria. **Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology**, v. 125, n. 2, p. 225-231, feb. 2000.

BUTTARELLI, R. F.; PELLICANO, C.; PONTIERI, F. E. Neuropharmacology and behavior in planarians: Translations to mammals. **Comparative Biochemistry and Physiology, Part C: Toxicology and Pharmacology**, v. 147, n. 4, p. 399-408, may. 2008.

CAMPBELL, W. C.; FISHER, M. H.; STAPLEY, E. O.; ALBERS-SCHÖNBERG, G.; JACOB, T. A. Ivermectin: a potent new antiparasitic agent. **Science**, v. 221, n. 4613, p. 823-828, aug. 1983.

CAMPBELL, W. C. Ivermectin: an update. **Parasitology Today**, v. 1, n. 1, p. 10-16, jul. 1985.

CAROLEI, A.; MARGOTTA, V.; PALLADINI, G. Proposal for a new model with dopaminergic-cholinergic interactions for neuropharmacological investigations. **Neuropsychobiology**, v. 1, n. 1, p. 355-365, nov. 1975.

EGERTON, J. R.; OSTLIND, D. A.; BLAIR, L. S.; EARY, D. H.; SUHAYDA, D.; CIFFELI, S.; RIEK, R. F.; CAMPBELL, W. C. Avermectins, new family of potent antihelmintic agents: efficacy of the B_{1a} component. **Antimicrobial Agents and Chemotherapy**, v. 15, n. 3, p. 372-378, mar. 1979.

ERIKSSON, K. S.; PANULA, P. Gamma-aminobutyric acid in the nervous system of a planarian. **The Journal of Comparative Neurology**, v. 345, n. 4, p. 528-536, jul. 1994.

FISHER, M. H.; MROZIK, H. Chemistry: Chemical properties, In: CAMPBELL, W. C. (Ed.). **Ivermectin and Abamectin**. New York: Springer-Verlag, 1989. p. 1-23.

FREITAS, R. M. C.; ALVES, S. N.; FARIA, M. A.; MELO, A. L. Effects of ivermectin on *Culex quinquefasciatus* larvae. **Revista do Instituto de Medicina Tropical de São Paulo**, v. 38, n. 4, p. 293-297, jul./aug. 1996.

GODDMAN, L. S.; GILMAN, A. G. **The pharmacological basis of therapeutics**. 8. ed. New York: Mc Graw-Hill, 2000. 1840 p.

HORVAT, T.; KALAFATIĆ, M.; KOPJAR, N.; KOVAČEVIĆ, G. Toxicity testing of herbicide norflurazon on an aquatic bioindicator species – the planarian *Polycelis felina* (Daly.) **Aquatic Toxicology**, v. 73, n. 4, p. 342-352, jul. 2005.

LENTZ, T. L. **Primitive Nervous Systems**. New Haven: Yale University Press, 1968. 148 p.

- MORITA, M.; BEST, J. B. Electron microscopic studies on planaria. II. Fine structure of the neurosecretory system in the planarian, *Dugesia dorotocephala*. **Journal of Ultrastructure Research**, v. 13, n. 5, p. 396-408, dec. 1965.
- NOEL, F.; MENDONÇA-SILVA, D. L.; THIBAUT, J-P. B.; LOPES, D. V. S. Characterization of two classes of benzodiazepine binding sites in *Schistosoma mansoni*. **Parasitology**, v. 134, n. 7, p. 1003-1012, feb. 2007.
- NISHIMURA, K.; KITAMURA, Y.; INOUE, T.; UMESONO, Y.; SANO, S.; YOSHIMOTO, K.; INDEN, M.; TAKATA, K.; TANIGUCHI, T.; SHIMOHAMA, S.; AGATA, K. Reconstruction of dopaminergic neural network and locomotion function in planarian regenerates. **Developmental Neurobiology**, v. 67, n. 8, p. 1059 – 1078, july. 2007.
- NISHIMURA, K.; KITAMURA, Y.; UMESONO, Y.; TAKEUCHI, K.; TAKATA, K.; TANIGUCHI, T.; AGATA, K. Identification of glutamic acid decarboxyl-ase gene and distribution of GABAergic nervous system in the planarian, *Dugesia japonica*. **Neuroscience**, v. 153, n. 4, p. 1103–1114, june. 2008.
- PASSARELLI, F.; MERANTE, A.; PONTIERI, F. E.; MARGOTTA, V.; VENTURINI, G.; PALLADINI, G. Opioid-dopamine interaction in planaria: a behavioral study. **Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology**, v. 124, n. 1, p. 51-55, sep. 1999.
- PRÁ, D.; LAU, A. H.; KNAKIEVIEZ, T.; CARNEIRO, F. R.; ERDTMANN, B. Environmental genotoxicity assessment of an urban stream using freshwater planarians. **Mutation Research**, v. 585, n. 1, p. 79-85, aug. 2005.
- SNYDER, G. L.; ALLEN, P. B.; FIENBERG, A. A.; VALLE, C. G.; HUGANIR, R. L.; NAIRN, A. C.; GREENGARD, P. **The Journal of Neuroscience**, v. 20, n. 12; p. 4480-4488, jun. 2000.
- STEFANO, G. B.; HIRIPI, L. Methionine enkephalin and morphine alter monoamine and cyclic nucleotide levels in the cerebral ganglia of the freshwater bivalve *Anodonta cygnea*. **Life Sciences**, v. 25, n. 3, p. 291-297, jul. 1979.
- SUPLAVILAI, P.; KAROBATH, M. The effect of temperature and chloride ions on the stimulation of [³H]flunitrazepam binding by the muscimol analogues THIP and piperidine-4-sulfonic acid. **Neuroscience Letter**, v. 19, n. 3, p. 337-341, oct. 1980.
- VENTURINI, G.; STOCHHI, F.; MARGOTTA, V.; RUGGIERI, S.; BRAVI, D.; BELLANTUONO, P.; PALLADINI, G. A pharmacological study of dopaminergic receptors in planaria. **Neuropharmacology**, v. 28, n. 12, p. 1377-1389, dec. 1989.
- WELSH, J. H. Evidence of a trophic action of acetylcholine in a planarian. **The Anatomical Record**, v. 94, n. 3, p. 421, mar. 1946.
- WELSH, J. H. Catecholamines in the invertebrates. In: BLASCHKO, H.; MUSCHOLL, E. (Eds.) **Handbook of Experimental Pharmacology**. Berlin: Springer-Verlag, 1972. p. 79-109.
- WELSH, J. H.; KING, E. C. Catecholamines in planarians. **Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology**, v. 36, n. 4, p. 683-688, oct. 1970.
- WELSH, J. H.; WILLIAMS, L. D. Monoamine-containing neurons in planaria. **The Journal of Comparative Neurology**, v. 138, n. 1, p. 103-115, jan. 1970.

WILLIAMS, M.; RISLEY, E. A. Interaction of avermectins with [3H]beta-carboline-3-carboxylate ethyl ester and [3H]diazepam binding sites in rat brain cortical membranes. **European Journal of Pharmacology**, v. 77, n. 4, p. 307-312, feb. 1982.

WILLIAMS, M.; YARBROUGH, G. Enhancement of *in vitro* binding and some of the pharmacological properties of diazepam by a novel anthelmintic agent, Avermectin B_{1a}. **European Journal of Pharmacology**, v. 56, n. 3, p. 273-276, jun. 1979.