Pakistan J. Zool., vol. 45(6), pp. 1593-1598, 2013.

Anthelmintic and Insecticidal Activities of *Verbascum thapsus* L.

Muhammad Riaz,[1]* Najmur Rahman[2] and M. Zia-Ul-Haq[3]
[1]*Department of Pharmacy, University of Swabi, Anbar, Swabi, Pakistan*
[2]*Department of Pharmacy, Kohat University of Science and Technology, Kohat, Pakistan*
[3]*The Patent Office,Karachi,Pakistan*

Abstract.- In present study, we investigated methanolic extracts of various parts of *Verbascum thapsus* L. for *in vitro* anthelmintic and insecticidal action. For anthelmintic activity *Pheretima posthuma* was used as test animal while 5, 10, 25, 50, 75 and 100 mg doses of *Verbascum thapsus* were applied. A remarkable anthelmintic activity was observed. The leaves and fruit extracts resulted in death of worms in 35 to 40 minutes. Keeping in view the mean paralytic time and mean death time, extract of leaves was most active followed by fruit extract then stem and the least root. *V. thapsus* extracts were studied for insecticidal activity using red flour beetle (*Tribolium castaneum*), its larvae and rice weevil (*Sitophilus oryzae*) at doses 1, 5, 10, 25, 50, 75, and 100 mg of extract. However, no significant insecticidal action was observed against the tested insects.

Keywords: *Verbascum thapsus* L., anthelmintic activity, insecticidal activity.

INTRODUCTION

Verbascum thapsus L. also known as Kharghwug (Murad *et al*., 2011), Ghordoughkaro (Hussain *et al*., 2007), Gidder Tambakoo (Qureshi *et al*., 2007), Tamakusak (Shinwari and Gilani, 2003), Khardhag (Sher, 2011), Jungle tambako and barbasco (Pullaiah, 2003), belongs to family *Scrophulariaceae*. It is erect biennial plant distributed in different Pakistani areas like Kurram Agency, Dir, Chitral, Swat, Gilgit, Deosai, Baltistan, Drass, Ladakh Hazara, Poonch, Kashmir, Baluchistan and Punjab (Shinwari and Gilani, 2003).

Since ancient times, it has been used as a medicinal herb. The leaves and flowers are reported to have expectorant and demulcent properties which are used to treat respiratory problems such as bronchitis, dry coughs, whooping cough, tuberculosis, asthma, and hoarseness (Grieve, 1981; Tyler, 1993, 1994). Leaf extracts of *V. thapsus* have been shown to be active against bovine herpes virus type 1, and showed slight antibacterial and antifungal activity (McCutcheon *et al*., 1992, 1993). Its methanolic extract has been shown to be effective against mosquito larvae (Gross and Werner, 1978).

* Corresponding author: pharmariaz@gmail.com
0030-9923/2013/0006-1593 $ 8.00/0

Various chemical constituents have been reported like saponins e.g. triterpene B, triterpene A, saikogenin A (Pascual Teresa *et al*., 1978); thapsuine B, hydroxythapsuine, thapsuine A, hydroxythapsuine A (Pascual Teresa *et al*., 1980); iridoid glycosides e.g. harpagide, verbascoside A, aucubin and isocatalpol and their various deravitives (Bianco *et al*., 1984; Seifert *et al*., 1985), and phenylethanoid glycosides (Warashina *et al*., 1991, 1992). Flavonoids (Souleles and Geronikaki, 1989) have also been reported from the plant. In present study, various parts methanolic extracts of *V. thapsus* were studied for their anthelmintic and insecticidal properties.

MATERIALS AND METHODS

Plant material (*Verbascum thapsus* L.) was collected from district Dir (L) Khyber Pukhtun Khwa, Pakistan. The plant parts were dedusted, chopped and dried in shade to prevent phytochemical degradation and to avoid fungus growth. Then the dried plant parts were soaked in methanol for fifteen days. The soaking and extraction process was repeated three times in order to elute compounds mixtures completely. The extracts were dried to get a gummy mass.

Anthelmintic activity

Anthelmintic activity was performed using *Pheretima posthuma* (earthworm) up to 4 to 8 cm in length and 0.2-0.3 cm in width collected from water

logged area of soil. The anthelmintic assay was carried out as per the modified method of Ajaiyeoba *et al*. (2001). The assay was performed in *vitro* using adult earthworm (*Pheretima posthuma)* owing to its anatomical and physiological resemblance with the intestinal roundworm parasites *Ascaris lumbricoids* of human beings for preliminary evaluation anthelmintic activity (Parida *et al*., 2010). Eight extracts 5, 10, 25, 50, 75 and 100 mg doses were used along with negative control distilled water. Yomesan® (Niclosamide) Bayer Pakistan Privet Limited was used as positive control in the same doses as extracts.

Insecticidal activity

V. thapsus extracts were studied for insecticidal activity using red flour beetle (*Tribolium castaneum*) and rice weevil (*Sitophilus oryzae*). Ten insects were taken from both red flour beetle and rice weevil per petri dish per single dose, with doses 1, 5, 10, 25, 50, 75, and 100 mg of extract dissolved in distilled water. The inner lining of each Petri dish was covered with the same size round cut filter paper and then an extract dissolved in distilled water was applied, waited for 10 minutes and then insects were added, observed for paralysis and death and repellent action. *T. castaneum* insects were taken from storage of old flour and were studied for these plants` extracts ten insect for each dose per Petri dish along with negative and positive control Mortein Coopex Powder (Permethrin 0.5% w/w) Reckitt Benckiser Pakistan Limited. *Sitophilus oryzae* also called rice weevil because of its habitat, we collect rice weevil from old rice in local rice godown Karachi. The same procedure was applied as for *T. castaneum.*

RESULTS AND DISCUSSIONS

Anthelmintic activity

Paralysis and death time of *P. posthuma* were the prominent observations along with excretion, motility and excitation. Because of easy availability, *P. posthuma* is used as suitable model organism during screening of anthelmintic activities of plant extracts. Leaves extract resulted in excitation of all worms but the excitation decreased within five minutes in high doses of extract and 50 mg, 75 mg and 100 mg paralyzed all worms within ten minutes died in 45 minutes, we observed sticky liquid resembling saliva within their petri dishes and their bodies were flaccid, indicating forceful expulsion. The stem exatrsct has anthelmintic action but milder than leaves of *Verbascum*, the same restless movement was observed as for leaves extract. However the minimum paralysis time of 45 minutes was observed with higher dose of 100 mg. Fruit extract was observed for the same strong action as leaves, paralysis was produced within 5 minutes of the drug application (100 mg) however death time (60 minutes) of fruit extract was longer then leaves extract (45 minutes). The root extract had weak anthelmintic action; paralysis was produced within 10 h and death after 24 h by larger doses while at smaller dose, no paralysis was observed even after 24 h. Overall, the extracts showed anthelmintic activity in a time-dependent and dose-dependent manner. The standard drug Yomesan® (Niclosamide) showed strong anthelmintic action (Table I).

Helminth infections are among the most widespread infections in humans, distressing a huge population of the world (Kosalge and Ravindra, 2009). Although few helminthes infections are lethal, most of them cause severe physical impairment. Children in developing countries are most prone to their effect although these infections can affect anyone (Peter *et al.*, 2007). The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases (Sondhi *et al*., 1994). Hence there is an increasing demand towards natural anthelmintics (Kosalge and Ravindra, 2009). Plant extract having anthelmintic potential usually have broad spectrum of action, high percentage of cure with a single therapeutic dose, free form toxicity to the host, environment friendly and are cost effective. The leaves of the plant had resulted death of worms in 35 minutes while standard drug in 40 minutes similarly the fruit or pods had comparable anthelmintic activity. A novel, comparable and remarkable activity was monitored that is anthelmintic activity of *V. thapsus*. Keeping in view the mean paralytic time and mean death time, we conclded that extract of leaves was most active followed by fruit extract then stem and root.

Table I.- Anthelmintic activity of fruit, leaves, root and stem of *V. thapsus* and standard drug Niclossmide.

Doses	Time after administration								
	1 min	5 min	10 min	15 min	30 min	1 h	2 h	4 h	24 h
Fruit									
Control	N	N	N	N	N	N	N	N	N
5 mg	0 +	2	2--	3--	4	D	-	-	-
10 mg	0 ++	2-	2--	3--	4	D	-	-	-
25 mg	0 ++	2--	4	4	4	D	-	-	-
50 mg	0 +++	3--	4	4	4	D	-	-	-
75 mg	0 +++	3-	4	4	4	D	-	-	-
100 mg	0 +++	4	4	4	4	D	-	-	-
Leaves									
Control	N	N	N	N	N	N	N	N	N
5 mg	0 ++	2	2	2	3	4	D	-	-
10 mg	0 ++	2-	2--	2---	3-	D	-	-	-
25 mg	0 +++	0++,S	3	4--	3	D	-	-	-
50 mg	0 +++	0+3	4,	3--	3--	D	-	-	-
75 mg	0 ++++	1-,S	4	3--	4--	D	-	-	-
100 mg	0 ++++	3,S	4	4S	4S	D, ↓	-	-	-
Root									
Control	N	N	N	N	N	N	N	N	N
5 mg	0 ++	0	2	2	2	N	N	N	A
10 mg	0 ++	0	2	2	2	N	N	N	A
25 mg	0 +++	0+	2	2	N	N	N	N	A
50 mg	0 +++	0+	0	2	2	2--	3	3--	D
75 mg	0 +++	1-	2--	2	2	3-	3-	3-	D
100 mg	0 ++++	2	2	2	2	3	3-	3-	D
Stem									
Control	N	N	N	N	N	N	N	N	N
5 mg	N	2	2	2	2	2	2	3	A/S
10 mg	1	0	2	2	2	2	2	2-	A/S
25 mg	0	0	2	2-	2---	3	4	4-	D
50 mg	0	0	0	3--	3--	3--	4	4-	D
75 mg	0	0	2	3--	4	D	-	-	-
100 mg	0	0	3	3, S	4, S	D	-	-	-
Stem									
Control	N	N	N	N	N	N	N	N	N
5 mg	0	2	2	3	4	D	-	-	-
10 mg	0	2	2	3	4	D	-	-	-
25 mg	0	2	2	3	4	D	-	-	-
50 mg	0 +++	2	2	3	4	D	-	-	-
75 mg	0 ++	2-	2--	3	4	D	-	-	-
100 mg	0 +++	2	2	3	4	D	-	-	-

A, Alive; D, death; E, edema; N, normal; S, secretions; 0, pontaneous motility; 1, moderate motility; 2, less motility; 3, reduce touch evoke response; 4, total paralysis; ↓, Size reduction; Intensity, +, high; ++, moderate high; +++, very high; -, low; --, moderate low; ---, very low.

Insecticidal activity

Stored-product insects may lead to post harvest losses, estimated from 9% in developed countries to 20% or more in developing countries (Phillips and Throne, 2010). *S. oryzae* and *T. castaneum* are major pests of stored grain products in the tropics (Rajasekharreddy and Rani, 2010). Therefore plant extracts were screened for

Table II.- Insecticidal activity of fruit, leaves, root and stem of *V. thapsus and permethrin.*

Dose	No. of survivor	*Tribolium castaneum*		*Sitophilus oryzae*	
		Time of onset of drug action (immobility time)	% Mortality	Time of onset of drug action (immobility time)	% Mortality
Fruit					
1 mg	10	2 h; 1P	0	-	0
5 mg	10	-	0	-	0
10 mg	10	-	0	-	0
25 mg	10	-	0	3 h; 1 P	0
50 mg	10	-	0	4 h; 1 P	0
75 mg	10	1 R	0	2 R	0
100 mg	10	2 R	0	2 h; 1 D, 1 R	10
Leaves					
1 mg	10	5 min; 1 P, 1 h; 1 D	10	10 min; 1 P	0
5 mg	10	5 min; 1 P, 1 h; 2 D	20	15 min; 1 P, 1 h; 1 D	10
10 mg	10	5 min; 01P 10 min; 1D	10	6 h; 2 P	0
25 mg	10	-	0	1 h; 1 D	10
50 mg	10	5 min; 1 P,12 h; 1 D	10	1 h; 1 P, 2h; 1 D	10
75 mg	10	2 R	0	1 h; 1 P, 1 D, 2 R	10
100 mg	10	10 min; 1P 5 min; 1 D, 3R	0	40 min; 1 D, 2 R	10
Root					
1 mg	10	1 h; 1 P, 2 h; 1 D	10	2 h; 1 P	0
5 mg	10	2 h; 1 P	0	-	0
10 mg	10	5 min; 1D, 6 h; 1 P	10	2 h; 1 D	10
25 mg	10	2 h; 1 P, 6 h; 1 D	10	2 h; 1 P	0
50 mg	10	5 min; 1 P, 1 h; 1 D	10	1 h; 1 P 1 h; 1 D	10
75 mg	10	1 h; 1 P, 2 h; 1 D, 3 R	10	4 h; 2 P, 5 h; 1 D	10
100 mg	10	1 h; 1 D, 2 h; 1 P, 4 R	10	2 h; 1 D, 2 R	10
Stem					
1 mg	10	-	0	-	0
5 mg	10	-	0	-	0
10 mg	10	-	10	-	0
25 mg	10	2 h; 01 D	10	2 h; 1 P	0
50 mg	10	6 h; 3 D	10	3 h; 2 P	0
75 mg	10	-	10	50 min; 1 P, 1 D	10
100 mg	10	24 h; 6 R	0	60 min; 1 D	10
Permethrin	100	235.9 µg/cm^2			100

R, repellent; P, paralyzed; D, died.

insecticidal activity against these two insects. Fruit extract at 100 mg showed 10% mortality against *S. oryzae* and 0% mortality against *T. castaneum* Table II. The activity results of leaves are presented in Table II. Table II shows the insecticidal activity of root and stem extract respectively. Permithrin as standard (Table II) showed 100% mortality at concentration of 235.9 µg/cm^2. Various extracts of the plant had no significant insecticidal action in our studies. Although the use of *V. thapsus* leaves and flowers have been reported for mites and ear infection (Lans *et al*., 2008). The plant leaves were reported for the presence of rotenone (Foster and Duke, 1990), which is naturally occurring compound commonly used as an insecticide in vegetable gardens (Betarbet *et al*., 2000) but the quantity might be very minute to exhibit insecticidal activity in our applied doses. Control of destructive insects in agriculture and forestry, storage of stocks, cattle-breeding, keeping of domestic animals and

hygienic sector increasingly poses serious problems. The greatest obstacle in the massive use of insecticides is their loss of efficacy due to resistance development in insects (Alexenizer and Dorn, 2007; Nadeem *et al.*, 2012). Plants may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals (Wink, 1993). The presence studies suggest that the plant extract may be fractionated for better results or the change of insects may also provide species specific insecticidal action.

ACKNOWLEDGMENT

The authors are thankful to Higher Education Commission of Pakistan for financial support.

REFERENCES

AJAIYEOBA, E.O., ONOCHA, P.A. AND OLARENWAJU, O.T., 2001. *In vitro* anthelmintic properties of *B. coriaceae* and *G. gynandra* extract. *Pharm. Biol*., **39**: 217-220.

ALEXENIZER, M. AND DORN, A., 2007. Screening of medicinal and ornamental plants for insecticidal and growth regulating activity. *J. Pestic. Sci.*, **80**: 205-215.

BETARBET, R., SHERER, T.B., MACKENZIE, G., GARCIA-OSUNA, M., ANOV, A.V. AND GREENAMYRE, J. T., 2000. Chronic systemic pesticide exposure reproduces features of Parkinson's disease nature. *Neuroscience*, **3**: 1301-1306.

BIANCO, A., GUISO, M., PASSACANTILLI, P. AND FRANCESCONI, A., 1984. Iridoid and phenylpropanoid glycosides from new sources. *J. Nat. Prod.*, **47**: 901-902.

BOWN, D., 1995. *Encyclopaedia of herbs and their uses.* Dorling Kindersley, London, UK.

CHEVALLIER, A., 1996. *The Encyclopedia of medicinal plants.* Dorling Kindersley, London, UK.

CHIEJ, R., 1984. *Encyclopaedia of medicinal plants.* MacDonald and Company Limited, Maxwell House, London, UK.

FOSTER, S. AND DUKE, J.A., 1990. *A field guide to medicinal plants, Eastern and Central N. America.* Houghton Mifflin Company, USA.

GRIEVE, M., 1981. *A modern herbal*. Dover publications Inc. New York, USA.

GRIEVE, M., 1984. *A modern herbal*. Penguin Books, London, UK.

GROSS, K. L. AND WERNER, P. A., 1978. The biology of Canadian weeds: *Verbascum thapsus* and *Verbascum blattaria*. *Canadian J. Pl. Sci*., **58**: 401-403.

HUSSAIN, F., SHAH, S.M. AND SHER, H., 2007. Traditionnal resource evaluation of some plants of Mastuj, District Chitral, Pakistan. *Pak. J. Bot.*, **39**: 339-354.

KOSALGE, S.B. AND RAVINDRA, A.F., 2009. Investigation of anthelmintic potential of some plants claimed by tribals satpuda hills. *Int. J. Pharm. Tech. Res*., **1**: 68-72.

LANS, C., TURNER, N. AND KHAN, T., 2008. Medicinal plant treatments for fleas and ear problems of cats and dogs in British Columbia. *Canad. Parasitol. Res*., **103**:889-898.

MABEY, R., 1988. *The New Age Herbalist*. Macmillan Publishing Company, New York, USA.

MCCUTCHEON, A. R., ELLIS, S. M., HANCOCK, R. E. W. AND TOWERS, G. H. N., 1992. Antibiotic screening of medicinal plants of the British Columbian native peoples. *J. Ethnopharmacol.*, **37**: 213-223.

MCCUTCHEON, A. R., ELLIS, S. M., HANCOCK, R. E. W. AND TOWERS, G. H. N., 1994. Antifungal screening of medicinal plants of the British Columbian native peoples. *J. Ethnopharmacol.*, **44**: 157-169.

MURAD, W., AHMAD, A., GILANI, S. A. AND KHAN, M. A., 2011. Indigenous knowledge and folk use of medicinal plants by the tribal communities of Hazar Nao forest, Malakand District, North Pakistan. *J. med. Pl. Res*., **5**: 1072-1086.

NADEEM, M., IQBAL, J., KHATTAK M.K. AND SHAHZAD, M.A. 2012.Management of *Tribolium castaneum* (Hbst.) (*Coleoptera: Tenebrionidae*) using Neem (*Azadirachta indica* A. Juss) and Tumha (*Citrullus colocynthis* (L.). *Pakistan J. Zool.*, **44:** 1325-1331.

PARIDA, S., PATRO, V.J., MISHRA, U.S., MOHAPATRA, L. AND SANNIGRAHI, S., 2010. Anthelmintic potential of crude extracts and its various fractions of different parts of *Pterospermum acerifolium* Linn. *Int. J. Pharma. Sci. Rev. Res*., **1**: 107-111.

PASCUAL TERESA, J.D., DIAZ, F. AND GRANDE, M., 1978a. Components of *Verbascum thapsus* L. I. Triterpenes. *An. Quim.*, **74**: 311-314.

PASCUAL TERESA, J.D., DIAZ, F. AND GRANDE, M., 1978b. Componentes Del *Verbascum thapsus* L. II. Aceite de las semillas. *An. Quim.*, **78C**: 107-110.

PASCUAL TERESA, J.D., DIAZ, F. AND GRANDE, M., 1980. Components of *Verbascum thapsus* L. III. Contribution to the study of saponins. *An. Quim. Ser. C.*, **76**: 107-110.

PETER, J.H., DAVID, H.M., ALAN, F., JACOB, K., SONIA, E.S., JEFFREY, D. S. AND LORENZO, S., 2007. Control of neglected tropical diseases. *N. Engl. J. Med.*, **357**:1018-1027.

PHILLIPS, T. W., JAMES, E. AND THRONE, J. E., 2010. Bio-rational approaches to managing stored - product insects. *Annu. Rev. Ent.*, **55**: 375-397.

PULLAIAH, T., 2003. *Encyclopedia of world medicinal plants.*

Sri Krishndevaria University Anantapur Regency Publication, New Delhi, India.

QURESHI, R. A., AHMED, M. AND GHUFRAN, M. A., 2007. Indigenous knowledge of some important wild plants as folk medicines in the area of Chhachh (distt. Attock) Punjab, Pakistan. *Electron. J. Environ. Agric. Fd. Chem*., **6**: 2500-2511.

RAJASEKHARREDDY, P. AND RANI, P. S., 2010. Toxic properties of certain botanical extracts against three major stored product pests. *J. Biopestic.*, **3**: 586-589.

RAVINDRA, S.K., 2009. Fursule investigation of *in vitro* anthelmintic activity of *Thespesia lampas* (Cav). *Asian J. Pharma. Clin. Res*., **2**: 69-71.

SEIFERT, K., SCHMIDT, J., LIEN, N.T. AND JOHNE, S., 1985. Iridoids from *Verbascum* species. *Planta Med.*, **5**: 409-411.

SHER, Z., KHAN, Z. AND HUSSAIN, F., 2011. Ethnobotanical studies of some plants of Chagharzai valley, district Buner, Pakistan. *Pak. J. Bot*., **43**: 1445-1452.

SHINWARI, Z.K. AND GILANI, S.S., 2003. Sustainable harvest of medicinal plants at Bulashbar Nullah, Astore (Northern Pakistan). *J. Ethnopharmacol*., **84**: 289-298.

SONDHI, S.M., SHAHU, R. AND ARCHANA, M., 1994. Antiamoebic and anthelmintic evaluation of heterocyclic compounds containing nitrogen and/or sulphur . *Indian Drug.,* **31**: 317-320.

SOULELES, C. AND GERONIKAKI, A., 1989. Flavonoids from *Verbascum thapsus*. *Sci. Pharm*., **57**: 59-61.

TURKER, A.U. AND CAMPER, N.D., 2002. Biological activity of common mullein, a medicinal plant. *J. Ethnopharmacol.,* **82**: 117-125.

TYLER, V.E., 1993. *The honest herbal*. Pharmaceutical Products Press, New York, USA.

TYLER, V.E., 1994. *Herbs of choice: the therapeutic use of phytomedicinals*. Pharmaceutical Products Press, New York, USA.

WARASHINA, T., MIYASE, T. AND UENO, A., 1992. Phenylethanoid and lignan glycosides from *Verbascum thapsus*. *Phytochemistry*, **31**: 961-965.

WARASHINA, T., MIYASE, T. AND VENO, A., 1991. Iridoid Glycosides from *Verbascum thapsus* L. *Chem. Pharm. Bull*., **39**: 3261-3264.

WINK, M., 1993. Production and application of phytochemicals from an agricultural perspective. In: *Phytochemistry and agriculture* (eds. T.A.V. Beek and H. Breteler), Vol. 34, Clarendon, Oxford, UK, pp. 171-213.

(Received 25 July 2013, revised 22 August 2013)