Environment Conservation Journal 13(1&2) 65-71, 2012
ISSN 0972-3099 (Print) 2278-5124 (Online)
Abstracted and Indexed

Aloe vera extract characterization and its protection against fenvalerate induced toxicity in *Heteropneustes fossilis*

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Received:15.04.2011 **Revised:26.06.2011** **Accepted: 28.02.2012**

Abstract

Pesticides constitute the important group of environmental pollutants since they are potent metabolic inhibitor. It is gradually being recognized that by use of natural herbs and herbal preparations in aquaculture we can deal with pesticidal problems. The present study aimed to evaluate the characterization of ethanolic extract of *Aloe vera* (*A.vera*) by IR , SEM and to investigate its immunomodulatory, antidiabetic and anticholesterol activity in *H. fossilis*after giving sublethal doses of fenvalerate. As blood is patho-physiological indicator, this study was performed to begin an assessment of the effect of fenvalerate on biochemistry of blood serum. In present study three sublethal doses of fenvalerate [0.25, 0.50 and 0.75 (p.p.m)] were given to test fish, *H fossilis* for 15 days. After 15 days same fishes were given *A.vera*leafextract [(*A.vera* A) 250, (*A.vera* B) 500 and (*A.vera* C) 1000 (mg/kg of body wt.)] for 30 days to find its protection after intoxication of fenvalerate. Fenvalerate found harmfull in all the three doses and *A.vera* was protective against doses of fenvalerate.

Keywords*: Aloevera, blood, fenvalerate, heteropneustesfossilis,Infra redspectroscopy, SEM*

Introduction

On one hand technological improvement improved quality of life, on the other hand it has created a number of hazards. One of the main effects of the application of pesticides and herbicides in agriculture is the pollution of aquifers (Anderson, 1982) because the pesticide is carried by irrigation water or rain along the soil profile (leaching).Fenvalerate is an insecticide that has been in use since 1976. It is a mixture of four optical isomers which h*A.vera*e different insecticidal activities. It is an ester of 2-(4-chlorophenyl)-3-methylbutyric acid and alpha-cyano-3-phenoxybenzyl alcohol,but lacks a cyclopropane ring. However, in terms of its insecticidalbeh*A.vera*iour, it belongs to the pyrethroid insecticides. It is most commonly used to control insects in food, feed, and cotton products, and for the control of flies and ticks in barns and stables.Several workers observed the effects of fenvalerate in invertebrates and vertebrates**.**

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Bradburyand Coats (1982)noticed effects of fenvalerate in brain and liver of bobwhite quail (*Colinusvirginianus*). It also has harmful effects on protein metabolism (Reddyand Bashamohideen, 1988) and blood of *Cyprinuscarpio*(Reddy*et al*., 2006).Ill effects by its deposition in lamb tissues were emphasized elaborately by Wszolek*et al*. (1981a).

Toxic chemicals discharged in environment gets into food chain and by entering into biological system they disturbed biochemical processes leading to health abnormalities. So it is very essential to develop uses some natural plant or animal products in aquaculture to minimize the contamination and also can be used to control various diseases.

A.vera is widely distributed Liliaceae plant in tropical regions and its le*A.vera*es, fresh juice, pulp, root are used for medicinal purpose. Several herbal preparations that can enhance the body's immune status are extensively being used in the indigenous system of medicines (Pittman, 1992) and also have been investigated for its antioxidant property.Khan, A.and HaleemD.J. (2006) noticed its protective effects on lindane induced hepatotoxicity and genotoxicity. Many studies were also carried out to

investigate the hypoglycemic effect of some plants used traditionally to treat diabetes beside identification of active ingredients, mode of action and safety (Grover *et al*., 2002). Can, *et al*, (2004) saw effect of *A.vera* leaf gel and pulp extracts on the liver in type-II diabetic rat models.Also synthetic hypoglycemic drugs cannot fully control glucose level as well as cause side effects prompting the patients stop taking the medication, *A.vera*is claimed to reduce blood glucose level and improve immune system against infection without toxicity(Ghannamand Kingston, 1986).

A clinical trial in diabetes mellitus patients has been done by Bunyapraphatsara *et al*. (1996)to observe antidiabetic activity of *A.vera*.Increased cholesterol level is also problem today as it causes heart and kidney diseases also medication are not fully worked. *A.vera* has positive effects on cholesterol control (Joshi and Dixit, 1986). Therefore, the present study was conducted to investigate the immunomodulatory,hypoglycemic and anticholesterol activity of an *A.vera e*xtract after toxicity of fenvalerate in *H. fossilis.*

Material and Methods

Le *A.vera*es of *A.vera* werewashed, epidermis was selectively removed and pulp in the center of the leaf was separated and homogenized and further extracted with 500 ml of ethyl alcohal. The filtrate was collected and evaporated to dryness under reduced pressure in a rotary evaporator. The residue was stored in dry sterilized containers at 4 ^{o} until further used.Characterisation of *A.vera* has been done to observe the characteristic properties by Infra Red Spectroscopy (IR) and Scanning Electron Microcope (SEM) in UGC-DAE, Atomic Research Centre, Devi AhilyaVishw*A.vera*idyalaya (D*A.VERA*V), Indore, [M.P], India.Live and healthy *H. fossilis* were collected from pond near santer near (MHOW). The fishes of *A.vera*erage length 23$\pm$ 3 and weight 100$\pm$10 gm were used. Brought them in laboratory, washed with 1% KMnO4 sollution for 5 minutes. After acclimatization of 15 days, 36 fishes were selected for experiment, irrespective of their sex. Prior to experiment, toxicity tests were conducted to determine the LC50 and safe concentration values of fenvalerate for 96 hours. The physico-chemical analysis of water was done according to standard methods published by A.P.H.A (1992).-Four aquarium were used, three of fenvalerate and fourth was of control group. Three different doses of fenvalerate in acetone as 0.25 p.p.m 0.50 p.p.m and 0.75 p.p.m were given to 9 fishes per acquaria for 15 days. Both control and treated fishes were sacrificed at time intervals and blood was collected serving caudal peduncle using a sharp knife. Blood was used for calculating W.B.C. Count by the *Neubauer*hemocytometerand serum was separated from the formed elements through the centrifugation at 3000 rpm for 15 minutes for Glucose estimation by GOD-POD method of Trinder.-Herbal extract doses for fishes were given by mixing with fish food, fenvalerate treated fishes were cured by three different does of *A.vera* as [(*A.vera* A) 250, (*A.vera* B) 500 and (*A.vera* C) 1000 (mg/kg of body wt.)] for 30 days in three separate acquaria.Both parameters were again taken to observe the therapeutic effects of *A.vera.*

Results and Discussion

Results of the analysis are given in table no. 1-4 while IR and SEM are dipicted in figures 2-9.Table 1 shows results of Fenvalerate intoxication and (Table 2, 3 and 4) *A.vera*treatment. WBC count decreased after administration of fenvalerate but increased after *A.vera* treatment, glucose values decreased as concentration of fenvalerate increased and continuous decrease in all values were observed to greater extent after *A.vera*treatment. Decrease in cholesterol after *A.vera* treatment noticed after fluctuations in fenvalerate doses in treated group.Health and disease are parameters of the effectiveness with which human and animals alike adapt to their environments. The herbals occupied a distinct place in life right from the primitive period till today. Recent upsurge in identifying non-dietary natural products associated with high degree of safety margin in cancer and hepatoprotective agents has been hailed by many investigators to be practically beneficial when the carcinogenic or hepatotoxic insult is mild to moderate. Our environment abound with lots of substances which can induce diseases include foodstuff, house whole chemicals, pesticides, industrial and agro-chemicals etc. This has made the screening of such chemicals necessary in order to monitor the degree of human exposure and how their in vivo toxic effects may be caused and prevented by use of animal or plant products. It is gradually being recognized that only by use of

natural herbs and herbal preparations in aquaculture we can deal with these problems. Treatments of bacterial diseases with various herbs h*A.vera*e been safely used widely in organic agriculture, veterinary and human medicine (Direkbusarakom, S. 2004).

Table 1: Effect of three doses of fenvalerate [0.25, 0.50 and 0.75 (p.p.m)] for 15 days

Parameters	**Control**	**0.25 p.p.m**	**0.50 p.p.m**	**0.75 p.p.m**
WBC count (per cumm)	4.800	3.130	2.980	1.990
Glucose (mg/dl)	48.5	36.4	29.7	24
Cholesterol (mg/dl)	210	303	198	240.2

Table 2:Effects of *A.vera* extract as [(*A.vera* A) 250, (*A.vera* B) 500 and (*A.vera* C) 1000 (mg/kg of body wt.)] for 30 days after 15 days Fenvalerate intoxication (0.25 p.p.m).

Parameters	**Control**	**Fen (0.25)**	***A.vera* (A)**	***A.vera* (B)**	***A.vera* (C)**
WBC Count (percumm)	4.80	3.130	3.74	4.440	5.680
Glucose (mg/dl)	48.5	36.4	32.2	27.4	25.1
Cholesterol (mg/dl)	210	303	198	195	191

Table 3:Effects of *A.vera* extract as [(*A.vera* A) 250, (*A.vera* B) 500 and (*A.vera* C) 1000 (mg/kg of body wt.)] for 30 days after 15 days Fenvalerate intoxication (0. 50 p.p.m).

Parameters	Control	Fen (0.50)	*A.vera* (A)	*A.vera* (B)	*A.vera* (C)
WBC Count (percumm)	4.800	2.980	4.530	4.970	5.790
Glucose (mg/dl)	48.5	29.7	26.4	22.1	19.3
Cholesterol (mg/dl)	210	198	195	192	188

Table 4:Effects of *A.vera* extract as [(*A.vera* A) 250, (*A.vera* B) 500 and (*A.vera* C) 1000 (mg/kg of body wt.)] for 30 days after 15 days Fenvalerate intoxication (0.75 p.p.m).

Parameters	Control	Fen (0.75)	*A.vera* (A)	*A.vera* (B)	*A.vera* (C)
WBC Count (percumm)	4.800	1.990	2.680	4.990	6.840
Glucose (mg/dl)	48.5	24	22	19.7	18.2
Cholesterol (mg/dl)	210	240.2	222	304	182

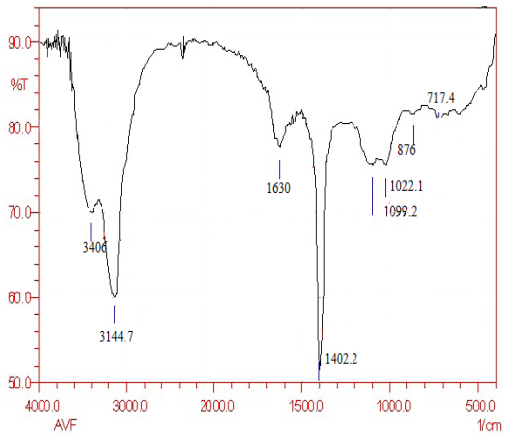

Fig.1: IR Spectroscopy results of*A.vera*h*A.vera*ing peak values in certain regions.

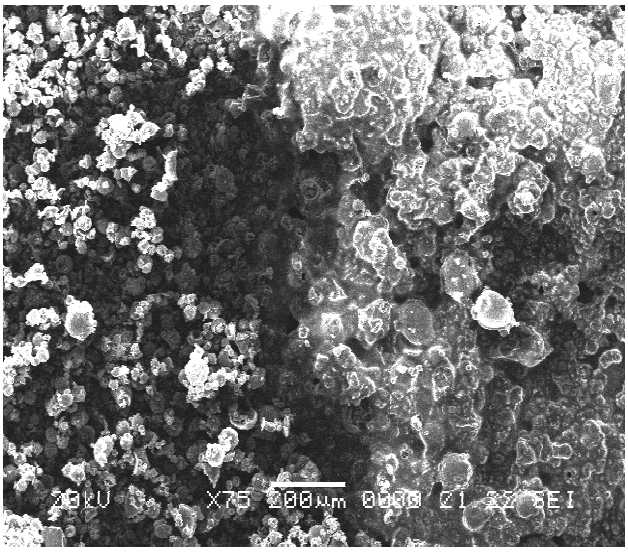

Fig. 2

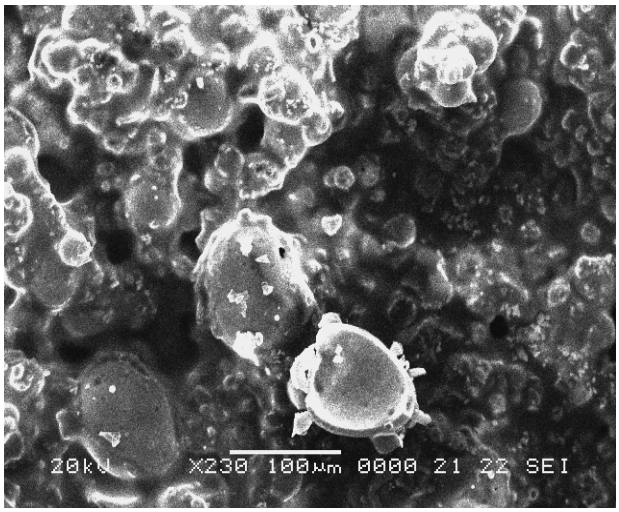

Fig. 3

Fig. 4

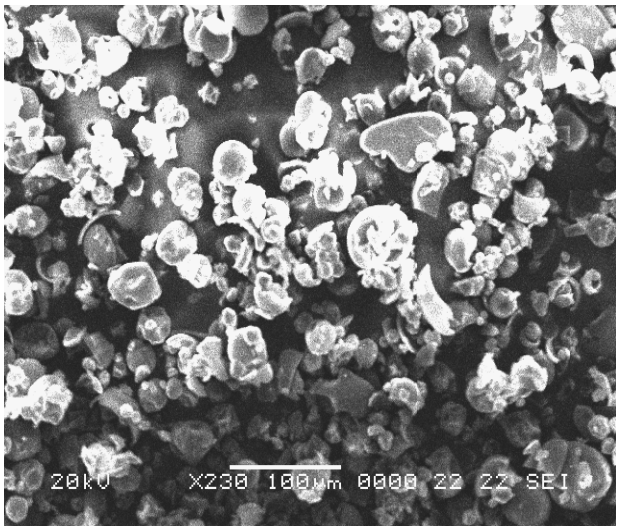

Fig. 5

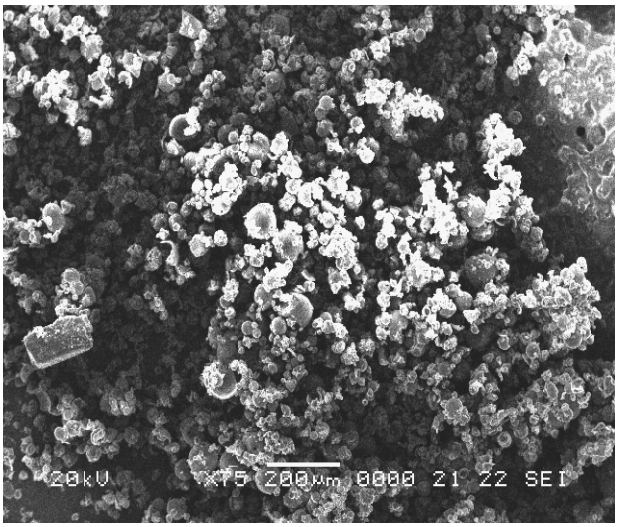

Fig. 6

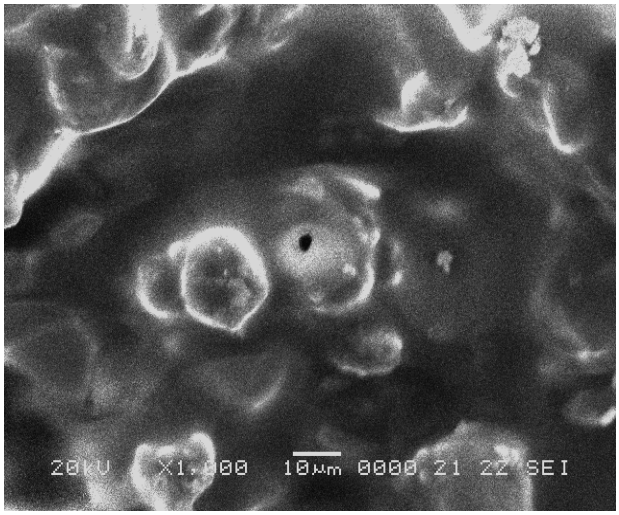

Fig.7

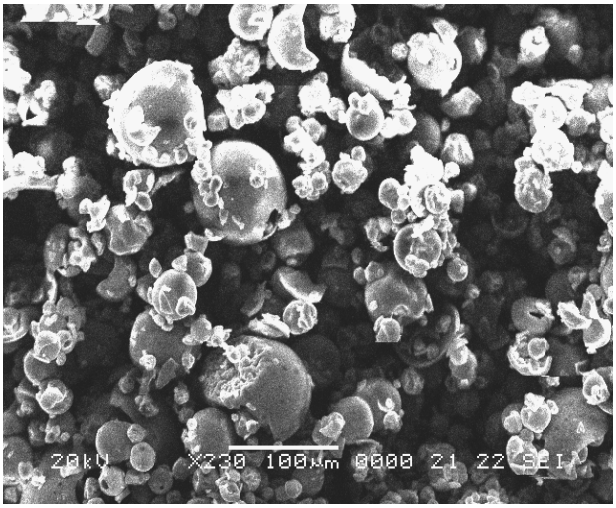

Fig. 8

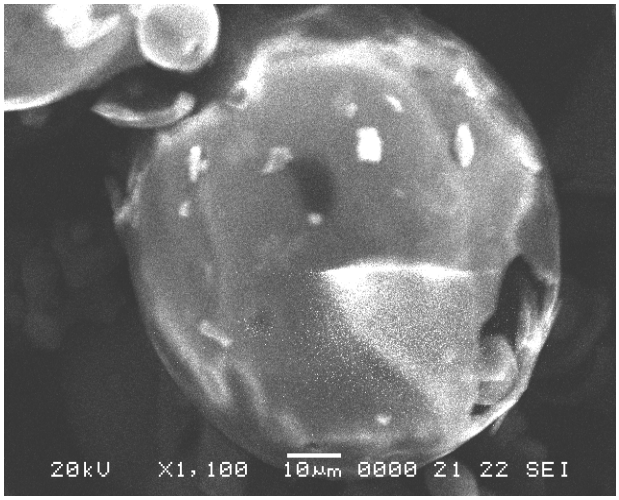

Fig. 9

Fig. 2-9 shows SEM structure of *A.vera.*

Infrared spectroscopy exploits the fact that molecules absorb specific frequencies that are characteristic of their structure. These absorptions are resonant frequencies, i.e. the frequency of the absorbed radiation matches the frequency of the bond or group that vibrates. Peak values of IR absorption pattern showed that *A.vera* contained phenolic –OH (alcoholic) and C=O (carbonyl) functional groups. The absorbtion pattern at 3406 and 3144.7 confirm the presence of OH functional group and at 1630, C=O is confirmed.SEM shows irregular and permeable round structure of *A.vera*that helps in react actively due to cell breaking with particle size range in between 50-700 nm.Decreased W.B.C Count due to fenvalerate concentration may be due to hypochromic microcytic anaemia lead to weak immune system. The present experiments revealed that *A.vera* extract has immuno-stimulatory action, stimulates the proliferation of stem cells as increased number of W.B.C found after*A.vera*treatment. *A.vera* is used as an adjuvant in conditions of immunodeficiency in cancer and to a limited extent in acquired immunodeficiency syndrome. Aloe contains a D- isomer polysaccharide called Acemannanwhich interjectsitself into all cell membranes results in an increase in the fluidity and permeability of themembrane allowing toxins to flow out and nutrients to enter the cell results inimproved cellular metabolism throughout the body and an overall boost in energy production. Acemannan also has direct effects on the cells of the immune system, activating and stimulating macrophages, monocytes, antibodies and T-cells and act as ant-leukamic agent (Sheets. *et al* 1991). It has been shown in laboratory studies to act as a bridge between foreign proteins and macrophages, facilitating phagocytosis (Stepanva*et al*.1977). This receptor site activation is a key component in boosting cell-mediated immunity which is deficient in HIV infection. A mixture of amino acids derived from Aloe enhanced the depressed phagocytic function of the white blood cells.(Yagi , 1987). Alexin B, a specific molecule species derived from Aloe, was shown to possess anti-cancer activity against lymphocytic leukemia. (Suzuki, 1979)Additional investigations revealed that another molecular species derived from Aloe, Aloctin-A, had anti-tumor activity, but the action was to bolster the immune system rather than a direct anti-tumor activity(Imanishi*et al*., 1981). Whatever the cause, low immunity and low white blood cell counts prevent the body from being able to h*A.vera*e an optimum response to infections and illness.In present investigation, decreased level of glucose (hypoglycemia) in fenvalerate treated fishes may be due to acute stress reaction , severe pancreatic and liver diseases or adreno cortical deficiency.*A.vera*doses also decreases the glucose but effect of *A.vera*ethanolic extract on glucose level is positive.Theantihyperglycaemic activity of *A.vera*was associated with an increase in plasma insulin, suggesting that the antihyperglycaemic activity of *A. vera* could be due to an insulinogenic activity of the extract. The increased levels of insulin observed in the present study indicate that the *A.vera*extract stimulates insulin secretion from the remnant b-cells and/or from regenerated b-cells. It increase carbohydrate utilization or enhancement of glucose uptake by muscles and increase activity of insulin secreting pancreatic B. cells as *A.vera*beh*A.vera*es like insulin. So when destruction of B. cells of islet of pancreas causes diabetes mellitus, *A.vera* is boon to reduce glucose. Fenvaleratetreatment results in cholesterol fluctuation. Decrease in cholesterol was due to its utilization to cope with energy demand to compensate the effect of toxicological stress. 60-80% of the total cholesterol is in esterified form. As esterification occur mainly in liver, the proportion of esterified cholesterol(so as total cholesterol) decrease in parenchymatous liver disease. According to Gupta (1974) cause of

hypocholesterolaemia was intestinal obstruction and of hypercholesterolaemia according to (Murrey, 1990) wasdue to impairment of liver and inhibition of enzymes, which converts cholesterol into bile acid. *A.vera* helps in lowering of cholesterol. Hypercholesterolemia commonly associated with coronary heart disease is correlated with an increase in the plasma LDL-cholesterol and a decrease in HDL- cholesterol concentration. Reduction in serum cholesterol caused by administration of *A.vera* can be attributed to a reduction in LDL+VLDL–cholesterol(that choke-heart). *A.vera* administration also increased the serum HDL-cholesterol (good cholesterol) ratio which is associated with a reduced incidence of atherosclerosis in humans (Joshi and Dixit, 1983). It thus seems to be an interesting agent which could be of use in the treatment of hypercholesterolemia. It improves and rebalance the quality of the blood and also contains B-sitosterol, which blocks cholesterol absorption in the body.

Conclusion

During investigations to modify the chemical structures of natural pyrethrins, a certain number of synthetic pyrethroids were produced with improved physical and chemical properties and greater biological activity.When pesticides enter aquatic systems, the environmental costs will be high. At times, pesticides are solely blamed for fish kills; however, in many cases, the indirect effects of pesticides, such as causing dissolved oxygen depletion, are the reason for the kill. Unintentional pesticide-related fish kills occur in India. Minimizing such contamination is possible by adjusting the maximum dose of pesticide and prior using a pesticide. Farmer should use pesticide only when necessary, wes should try to use less toxic pesticides. Human beings can be cured by various remedies but this is not possible in fishes due to speeking difference. Indirectly pesticides are taken by human beings by eating fishes as food.

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