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ORIGINAL ARTICLE

EFFECT OF *CISSUS QUADRANGULARIS* ON THE BIOCHEMICAL PARAMETER IN THE FRESH WATER FISH, *OREOCHROMIS MOSSAMBICUS*

***K.Aruldoss, R.Kannan and S. Divya**

Department of Zoology, Periyar Arts College, Cuddalore-607 001,Tamilnadu

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ABSTRACT

Cissus quadrangularis L.belongs to the family vitaceae and is an indigenous medicinal plant of India. The present study was aimed to investigate the effect of *Cissus quadrangularis* on glycogen content in fresh water fish, *Oreochromis mossambicus* at 7 and 14 days. The glycogen content was slightly increased in the liver, gill and muscle tissue of fish exposed with *Cissus quadrangularis* when compared with control fish. The present study concludes that *Cissus quadrangularis* is an beneficial to the growth and development of fishes.

Keywords: *Cissus quadrangularis, Oreochromis mossambicus, Tissues*

1.INTRODUCTION

Plants having medicinal property have been a major source of therapeutic agents for alleviation of complete cure of many human diseases since times immemorial. In India, the medicinal Plants are used widely by all sections of people either directly as folk remedies or in different indigenous system of medicine or indirectly in the pharmaceutical preparation of modern medicines.The medicinal plant based industry is growing at the rate of 7.15% annually. India, being a rich reservoir of natural resources has immense potential to capture the world markets in the area of medicinal and aromatic plants and their products . According to national health experts, more than 200 different plants are used for medicinal preparations for both internal and external use in India alone.

Cissus quadrangularis L.belongs to the family vitaceae and is an indigenous medicinal plant of India: it is known as " asthisnghara" in Sanskrit, Meaning which will strengthen the bones.This plant has been in safe use for countries. One that heals bones and joint problem, relief from pain without side effects can aid in the healing of overuse injuries, help solve gastrointestinal issues such as ulcers or acid reflux, is full of antioxidants and vitamins real plant that has been use and is recorded in ancient ayurvedic texts and has been applied by modern medicine to be completely safe. It gives pain relief and anti inflammatory effect at the level of aspirin or ibuprofen without any side effect associated with the drugs (Panthong *et al*., 2007).Plant extract of *Cissus* is like many such products. There are a vast number of phytonutrients that work synergistically together to produce an effect much greater than the whole. The tendril shoots and young leaves are used in various food preparations. The juice of the plant is said to be curative in scurvy. The plant contains high amounts of vitamin C, carotene and anabolic steroid substances. The plant has been used as an asthelmintic, antiseptic, digestive tonic, analgesic and treatment for scurvy and asthma.

Many studies have been shown that hormone replacement therapy in postmenopausal women may increase the risk of breast cancer, heart disease and many women are looking at alternative to estrogen to help prevent osteoporosis. Although there appears to be no published research showing that *Cissus* increases bone density in osteoporosis, or helps prevent the disease, the disease, the fact that the herb speeds recovery of fractures suggests that it may increase bone density as well. Besides the above -mentioned properties of *Cissus*, the plant is also rich in the vitamins antioxidants vitamin C and beta-carotene. As analyzed, *Cissus quadrangularis c*ontained ascorbic acid 479 mg. and carotene 267 units per 100g of freshly prepared paste in addition to calcium oxalate (Chidambara Murthy *et al*., 2003).

Cissus also possess the properties on a mg basis comparable to aspirin or anti- inflammatory drugs like ibuprofen. *Cissus guadrangularis* constituents are one of the ingredients of an Ayurvedic preparation, 'Laksha Gogglu", which has been

****Corresponding author:*** *Dr. K.Aruldoss, Department of Zoology, Periyar Arts College, Cuddalore-607 001,Tamilnadu*

proved to be highly effective in relieving pain, reduction of swelling and promoting the process of healing of the simple fractures as well as in curing the allied disorders associated with fractures (Panda 1990). The mechanism through which *Cissus* extracts its analgesic and anti- inflammatory properties has not been well characterized. It may act centrally but preventing the conversion of arachidonic acid to inflammatory prostaglandins. It is one of the most widely used ingredients in alternative medicine (Ayurveda) for the treatment of piles, anorexia, indigestion, chronic ulcers, asthma, wounds and in augmenting fracture healing process (Rajpal, 2002).

Traditional medicine in many areas of the world relies of the use of a wide variety of plant species in Africa. Phototheraphy still plays an important role in the management of diseases mainly among population with very low income. *Cissus quadrangularis* Linn (Vitaceae) originate from India and Malaysia, grows in Savannah areas in Africa (Cameroon, Mali, Mauritania, Senegal, Somalia and Chad). In traditional medicine, the plant is used to treat hemorrhoids, Anorexia, indigestion, and asthma, (Rajpal, 2002). In sahelian areas particularly *C. quadrangulais* is used in the treatment of Sickle cells, Syphilis, gonorrhea. Fractures, colds, pains, malaria, abscess, asthma and as an analgesic (Arbonie, 2000). The plant is also used in Cameroon for the treatment of epilepsy (Personal communications). Chemical Studies have shown that the presence of sterols, steroids, tannins, flavonoids, carotenes, ascorbic acid linoleic acid in *C. quadrangularis* (Sen 1996). Biologically active compounds from natural sources have always been a great interest for scientists working on infectious diseases. The biochemical changes have been influenced by experimental diet. (*Cissus quadrangularis)* extract increase in the metabolism and glycogen contents of the tissues. Enzymes at cellular level lead to enhance the growth, and longevity of the organisms.

Carbohydrate metabolism plays an important role in energy yielding process. Glycogen, being the chief source of energy for the fish, is the first metabolite to be affected by any stress. Glucose, a reliable source of energy, is present in almost all tissues. Glucose is the most important function of carbohydrate and it serves as the major metabolic fuel. Rate of lactic acid production is considered as an index of physiological conditions of glucose metabolism. Free amino acids would also serve as precursors for energy production under stress and for the synthesis of required proteins to face the stress. The assessment of the protein and total amino acid content can be considered as a diagnostic tool to determine the physiological phase of the cell. Carbohydrate supplies a major portion of energy to the living system.

2.MATERIALS AND METHODS

Procurement and rearing of experimental fishes

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Oreochromis missambicus commonly called African Mouth breeder is widely distributed in the freshwater of India. *Oreochromis mossambicus* was collected from the fish farm located in puthur, 40 km away from the Periyar Arts College, Cuddalore-1. The collected fishes without least disturbance were transported in polythene bags filled half with water. About 50 fishes were put in each bag and water was well aerated, using pressurized air from a cylinder. This mode of transit proved successful, since there was no mortality in all consignments throughout the course of this study.

The fishes brought to the laboratory were acclimatized in fibre aquarium for a fortnight before they were used for the experiment. The fish tanks were kept free the fungal infection by washing with potassium permanganate solution. The fish were disinfected with 0.1% potassium permanganate solution and were maintained for three weeks in well aerated tap water. Test stress, physical damage and mortality. The injured, severely diseased, abnormal and dead individuals were discarded. Feeding was discontinued two days prior to the commencement of the experiments to reduce the additive effects of animal excreta in the test through (Arora *et al*., 1972). The fishes were exposed to plant extract and control for the period of 7 and 14 days. A control group was maintained with identical environment. The plant extract with water and normal water was renewed every day. The fish were sacrificed from both experimental and control groups on 7 and 14 days of exposure periods.

Preparation of Fish Feed

For the present study, two different types of feeds were prepared following Hardy's Square Method (1980)

I. Control feed - Feed 1
II. *Cissus quadrangularis* plant extract mixed feed - Feed 2

The control feed was a standard based diet. It was prepared as a mixture of Rice bran (6.30%), Tapioca flour (21.30%), Groundnut oil cake (13.50%), Wheat flour (15.60%), Corn flour (15.20%), Rice pori (4.20%), Soya meal (22.70%), Vitamin and mineral mix (1.10%). The proximate composition of all the dried, powdered ingredients was analyzed according to AOAC procedures (1990).

Feed pellets were prepared by following the methods of Bindu and Sobha (2004). Appropriate quantities of finely powdered ingredients were weighed and mixed thoroughly by adding water. The dough thus prepared was stem cooked for 30 min in a pressure cooker. The cooled dough was fortified with vitamin and mineral mix 1% and was palletized using a hand pelletizer. The extruded pellets were dried overnight in a hot air oven at 60^{o}C. Proximate analysis of diets was carried out using standard methods (AOAC, 1990). (Table 1.).

Table 1 Proportion of ingredients and proximate composition of the feed percentage composition (g)

Feed ingredients	Quantity (100 g)	Quantity (200 g)
Groundnut oil cake	13.500	27.00
Tapioca flour	21.300	42.60
Wheat flour	15.600	31.200
Corn flour	15.200	30.400
Rice bran	6.300	12.600
Soya Meal	22.700	45.400
Rice Pori	4.200	8.400
Vitamins/ minerals (Riboflavin)	1.100	2.200

Plant extract preparation

Collection of Plant Material

One medicinal plant the *Cissus quadrangularis* that is described in traditional Indian medicine was chosen in this study in consultation with a practitioner of traditional Sidda medicine in the Union territory of Pondicherry. The availability of the plant material in sufficient amounts was also considered in chosen them. The plant chosen were *Cissus quadrangularis* (Vitaceae). The plants powder collected from the K.S.R. HERBAL PRODUCTS 2, Sanakkiya Nagar, Manjakuppam, Cuddalore (MI. NO. 330181100346E). The plants materials thus obtained were shade dried and powdered. The powdered material was stored in air tight containers.

Preparation of Extracts

The plant material was extracted with four solvents with different polarities Starting with Hexane (Polarity index 0) followed by Chloroform 50ml (Polarity index 4.1), Ethyl Acetate 50ml (Polarity index 4.4) and Methanol 50ml (Polarity index 5.1),and 250ml of distilled water added. The one plant materials (100gm) were extracted sequentially with 1:1:1 solvent to dry weight ration for 24 hours on a shaker at 200 r.pm. The extracts were then filtered through a Whatman filter paper and the filtrate collected in glass beakers.

The plant material was then re-extracted several times for maximum efficiency. The filtrates were dried in a Rotor Vapor Aspirator by applying vacuum and the solvents recovered were reused for extraction. The extracts were dried further by keeping them in vacuum desiccators. They were then stored in airtight containers at 4^{o} C and used for further analyses.

Experimental Procedure

Oreochromis mossambicus weighing 20-25g were divided in to 3 groups and stocked at random into 3 different concrete tanks each tank was assigned a specific type of feed.

The following experimental groups were conducted in the freshwater fish *Oreochromis mossambicus* for the period of 7 and 14 days.

Group I - Fish exposed t tap water and fed with feed -1 (Control feed1)
Group II - Fish exposed to tap water and fed with feed 2 (*Cissus quadrangularis* extract mixed for 7 days
Group III - Fish exposed to tap water and fed with feed 2 (*Cissus quadrangularis* extract mixed for 14 days

Estimation of Glycogen in the tissues

The calorimetric micro method of Kemp and Kits Van Heijininger (1954) was employed for the Quantitative estimation of glucose and glycogen. The tissues were isolated and homogenized in 5.0 ml of 8 per cent methanol and centrifuged at 3,000rpm for 1.5 minutes. The supernatant containing free glucose was decanted into calibrated test tube. The residue was set apart for the quantitative estimation of glycogen. For the estimation of glycogen, the residue left after methanol extraction was homogenized in 5.0ml of deproteinizing silver sulphate solution (0.5g TCA and 100mg AgSO4 in 100ml of distilled water) and heated at 100^{o}C over water bath for 15 minutes. The mixture was cooled and made up to 5.0ml with deproteinizing solution once again and later centrifuged at 2,000 rpm for 10 minutes. The clear supernatant was collected for the estimation of glycogen.
Quantitative Estimation of Glycogen

1.0ml of respective sample was taken in a separate test tube and3.0ml of concentrated sulphuric acid was added to it. The mixture was heated in a boiling water bath for 6.5 minutes and subsequently cooled and developed color was measured in a grating spectrophotometer (Cecil, Model CE 33730 against the reagent bland (3.0 ml concentrated sulphuric acid) at 520 nm. The quantities of glycogen present in the respective samples were read from the standard graph drawn previously from known quantities of glycogen. The glycogen values were expressed as mg/g wet weight of tissue.

Statistical Analysis

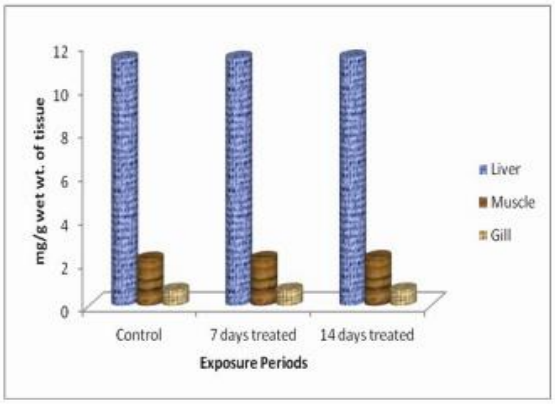
The values are expressed as mean $\pm$ SE. Data were statistically analysed by Analysis of Variance (ANOVA) along with Duncan's Multiple Range Test (DMRT) (Duncan, 1955) which was applied to find out significant difference between various treatment means and control means for the observed parameters.

3.RESULTS

Level of glycogen content in the liver tissue

The glycogen content in the tissue of liver was normal in fish fed with feed I (group I) when compared to fish fed with feed II (group II & III). The change over in the content was 0.176 and 0.616 for 7 and 14 days respectively. There was significant changes in the fish fed with feed 2 *Cissus quadrangularis* (group II & III) when compared to feed 1 (group I) for 7 and 14 days. (Fig .1).

Fig. 1 Glycogen level of liver, muscle and gill tissues in the control and experimental fish, *Oreochromis mossambicus*



Level of glycogen content in the muscle tissue

The glycogen content in the tissue of muscle was normal in fish fed with feed I (group I) when compared to fish fed with feed II (group II & III). The change over in the content

was 1.40 and 3.27 for 7 and 14 days respectively. There was significant changes in the fish fed with feed 2 *Cissus quadrangularis* (group II & III) when compared to feed 1 (group I) for 7 and 14 days. (Fig .1).

Level of glycogen content in the gill tissue

The glycogen content in the tissues of gills was normal in fish fed with feed I (group I) when compared to fish fed with feed II (group II & III). The change over in the content was 0.421 and 1.125 for 7 and 14 days respectively. There was significant changes in the fish fed with feed 2 *Cissus quadrangularis* (group II & III) when compared to feed 1 (group I) for 7 and 14 days. (Fig .1).

4.DISCUSSION

Carbohydrate supplies a major portion of energy to the living system. Disturbance in carbohydrate metabolism is the most outstanding biochemical lesion arising from the action of toxic compounds (De Bruin, 1976). Glycogen, the food reserve is utilized more to meet the extra demand of energy during stress condition which leads to the decrement.

In the present study, *Oreochromis mossambicus* exposed to plant extract feed 2 (group II) shows a significant near to normal in the glycogen level of liver muscle, and gill tissues for 7 and 14 days of exposure periods. Several investigations have been made on the effect of plant extract on the glycogen and glucose levels of fishes (Sharma *et al*., 2004, Sivaram *et al*., 2004, Immanuel, *et al*., 2007). Liver and muscle are two active sites where storage and metabolism of glycogen take place.

Shoba Rani (2000) has reported a significant of glucose contents in the liver, muscle and gill of *Tilapia mossambica* exposed to plant extract. Aruldoss and Indra (2012) has suggested that the significant increase in fish tissue of glycogen after treatment of two medicinal plants.

Dietary intake of feed 2 mixed with a significant increase in tissue glycogen when compared with fed with normal feed (feed 1). The glycogen content are significantly increased in plant extract fish fed with feed 2 than fish fed with feed 1 and it indicates that *Cissus quadrangularis* mixed feed (Feed 2) is more effective than Control feed (feed 1). Muthulingam (2002) has reported oral administration of chloroform and ethyl acetate extract of *Astercantha longifolia* and also *Silymarin t*o carbon tetra chloride treated rats shows significant increase in glycogen content in liver and kidney when compared to CCL_4 alone treated rats.

Digvijay singh and Ajay Singh, (2002) have reported that the aqueous latex extracts of Euphorbia. *royleana* and *Jatropha gossypifolia*. They have further reported the recovery of all the above parameters in *Channa punctatus* after withdrawl from *Euphorbia royleava*. Mohamed Salahy and Abd Allah Mohamound (2003) have reported hypoglycemia, hypolepidaemia, hypocholesterlaemia, hypotriglyceridaemia, promotion of glycogensis and lipogensis in white muscle of carnivorous fish, *Chrysichthyl auratus* after oral administration of garlic juice (Allium Satiuvm). Ascorbic acid acts as a protective agent against aldrin pollution in *Channa punctatus* in which mortality is reduced after addition of ascorbic acid in diet (Agrawal and Mahajan, 1978).

Sahin *et al*. (2002) have reported that higher dietary vitamin E resulted in a decrease in serum glucose, urea, triglycerides and cholesterol concentration while protein concentration increased when dietary vitamin E was increased. An improvement in the Level of Total Protein and glucose level near to normal has been reported in the blood of lead intoxicate wistar rat supplemented with Vitamin C and *Silymarin (*Shalan *et al*., 2005).Sharma et. al, (2004) have reported that the *Spirulina* feed improves toleration of fish towards methyl red, which may be ascribed to their better health and increase in total protein content. Many observations have shown that during recovery after stress the lactate content of fish muscle reduces as the glycogen content increases (Wardle, 1987). Thus feed 2 mixed with *Cissus quadrangularis* has more antioxidant properties then feed 1. Several herbs have been tested for their growth promoting activity in aquatic animals (Sivaram *et al*., 2004; Immanuel *et al*., 2007). The present study also demonstrated that diets supplemented with acetone extract from *C.dactylon, A. marmelos, W.somnifera and Z. officinale* enhanced growth in *O. mossambicus* fed for 45 days .Among the tested diets, *Z officinale (*H_4) Enhanced growth by >40% compared with the fish fed the plant extract diets, and this is also consistent with the work carried out by Citarasu *et al*.(2002) in *E. tauvina*, and Manju and Nair (2004) were aqueous extracts from leaf, stem and root of *A. marmelos* enhanced growth of *Anabas testudineus* (Bloch).Herbal extracts have been shown to reduce mortality against pathogenic challenges in fishes (Kim *et al*., 1999: Jian and Wu 2003).

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6.REFERENCES

Agarwal, V.S. 1997. Enumeration of Indian Drug species, *African Journal of Biomedical Research.* 1:276-277.

AOAC 1990. Official Methods of Analysis of the Association of official Analypical Chemists. 17th Edn., AOAC, Arling to Virginia.

Aruldoss, K and Indra, N 2014. Impact of lead and influence. of different feeds on Carbohydrate metabolis in the muscle Carbohydrate metabolis in the muscle tissue of fresh water fish, *Oreochromis mossambicus. Int. J. Mod. Res. Rev*. Vol. 2(10 47-51.

Chidambara Murthy, K.N., Vanitha, A., Mahadev SWAMY,M., Ravisankar, G.A. 2003. Antioxidant and antimicrobial activity of *Cissus quardrangularis* L. *Journal of medicinal food.* V.6:2-3.

Citarasu, T., Michael Babu, M., Raja Jeya Sekar, R. and Peter Marian, M. 2002. Developing artemia enriched herbal diet for producing quality larvae in *Penaeus monodon*, Fabricius. *Asian Fisheries Science* 15,21-32.

Citarasu, T., Sivaram, V., Immanual, G,. Namita, Rout, Murugan V. 2006. Influence of selected Indian immunostimulant herbs against white spot syndrome virus (WSSV) infection in black shrimp, Penaes monodon with reference to haematological, biochemical and immunological changes. *Fish Shellfish Immunol*.21, 372-384.

Citarasu, T., Venketaramalingam, K., Raja Jeya Sekar, R., Micheal Babu, M.&Marian, M.P. 2004. Influence of the antibacterial herbs, *Solanum trilobatum*, *Andrographis paniculata* and *Psoralea corylifolia* on the survival, growth and bacterial load of *Penaeus monodon* post larvae. *Aquaculture International* 11,583-595.

Immanuel,G., Citarasu, T., Sivaram, V.,Michael Babu,M. and Palavesam, A.2007. Delivery of HUFA , Probionts and biomedicine through bioencapsulated Artemia as a mean to enhance the growth and survival and reduce the pathogenecity in shrimp *Penaeus monodon* postlarvae. *Aquaculture International* 15,137-152

Jian, J. and Wu, Z.2003. Effect of traditional Chinese medicine on nonspecific immunity and disease resistance of larvae of yellow croaker *Pseudosciaena crocea* (Richard-son). *Aquaculture* 218: 1-9.

Kemp, A. and J.M. Kitsvan Heijhinger 1954. A colorimetric micromethod for the determination of slylogen in tissues. *Biochem .J*.,56: 646-648

Kim, K.H., Hwang, Y.J. and Bai, S.C. 1999. Resistance to *Vibrio alginolyticus* in juvenile rock fish (*Sebastes schlegeli)* fed diets containing different doses of aloe. *Aquaculture* 180, 19-21.

Manju, K.and Nair, G.R.J. 2004. Effect of Indian beal tree extract of protein metabolism in the fish *Anabas testudineus. Journals of Ecotoxicology and Environmental Monitoring* 14, 221-226.

Muthulingam, M. 2002. Studies on the curative efficacy of *Astercantha longifolia* l., Nees (Acanthaceae) on carbon tertrachloride indued hepatotoxicity in rats. Ph.D., Thesis, Annamalai University India.

Panda, J. 1990. Anabolic siddha effect of *cissus.* Res Ayurvedic Siddha, 11,17.

Panthong, A., Supraditaporn, w., Kanjanapthi, D., Taesotikul., Reutrakul,V. 2007. Analgesis, anti-inflammotory and venotonic effect of *Cissus quadrangularis* Linn. *J. Ethnopharmacol Mar*21:26-47.

Rajpal, V. 2002. Standaridization of Botanicals, Vol.77-81. Eastern publishers, New Delhi, India.

Sahu, S., Das, B.K., Mishra, B.K., Pradhan, J and Sarangi N. 2007. Effet of Allium *sativum* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila. Journal of Applied Ichthyology* 23,80-86.

Sharma A, Deo AD, Riteshkumar ST, Chanu TI, and Das, A. 2010. Effect of Withania Somnifera (L.Dunal) root as a feed additive on immunological parameters and disease resistance to *Aeromonas hydrophil* in *Labeo rohita* (Hamilton) Fingerlings. *Fish Shellfish Immunology*. 29(3) : 508 -512.

Sivaram, V., Babu , M.M., Immunel, G., Murugadass, S., Citarasu, T. and Marian M.P., 2004. Growth and immune response of juvenile greasy groupers (*Epinephelu stauvina*) fed with herbal antibacterial active principle supplemented diets against *Vibrio harveyj* infections. *Aquaculture* 237,9-20.

Vasudeva R.Y., Chakrabarti R. 2005. Dietary Incorporation for *Achyranthes aspera* seed influences the immunity of common carp *Cyprinus carpio, Indian J. Anim* , Sci.75 200,p.1097-1102.

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