



HISTOLOGICAL STUDIES ON THE *CISSUS QUADRANGULARIS* PLANT EXTRACT TREATED FISH *OREOCHROMIS MOSSAMBICUS* PETER

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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 histological changes of normal and *cissus quadrangularis* plant extract treated fresh water fish, *Oreochromis mossambicus* tissues samples (gill, liver and muscle) of *Oreochromis mossambicus*, at 7, 14 and 21 days. The fish exposed with *Cissus quadrangularis* when compared with control fish. The present study concludes that *Cissus quadrangularis* is a beneficial to the growth and development of fishes.

KEYWORDS : *Cissus quadrangularis*, *Oreochromis mossambicus*, Tissues (gill, liver and muscle)

INTRODUCTION

Indian medicinal plants are the essence of Ayurveda and Ayurvedic treatments. When used judiciously and clocking with the basic principles they produce miraculous effects. Their role cannot be confined to mere curative of disease but they also used being of human body. Hence, Ayurvedic drugs are rightly called the elixirs of life. Ayurvedic Herbs played important role in Ayurvedic treatment, from ancient time to this most modern time. Throughout the ages, humans have relied on nature for their basic needs, for the production of food, shelter, clothing, transportation, fertilizers, flavours and fragrances, and medicines (Cragg and Newman, 2005).

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 (Singh, et al., 2003). 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 used and is recorded in ancient ayurvedic texts and has been applied by modern medicine to be completely safe. Plant extract of *Cissus quadrangularis* L. 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 tendrils 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 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* contained 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 quadrangularis* constituents are one of the ingredients of an Ayurvedic preparation, 'Laksha Gogglu', which has been 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. 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 (Agarwall, 1977 and Rajpal 2002).

Traditional medicine in many areas of the world relies on the use of a wide variety of plant species in Africa. Phototherapy 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) (Arbonier, 2000). In traditional medicine, the plant is used to treat hemorrhoids, Anorexia, indigestion, and asthma, (Rajpal, 2002). In sahelian areas particularly *C. quadrangularis* is used in the treatment of Sickle cells, Syphilis, gonorrhoea. 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* (Marty, et al., 2003). Biologically active compounds from natural sources have always been a great interest for scientists working on infectious diseases. *Cissus quadrangularis* is used by common folk in India to hasten the fracture healing process. Phytochemical analyses of *Cissus quadrangularis* reveal a high content of ascorbic acid, carotene, phytosterol substances and calcium (Harikrishnan et al. 2003). Shirwaikar et al., (2003) have studied that the antiosteoporotic effect of ethanol extract of *Cissus quadrangularis* Linn. on ovariectomized rat.

Medicinal plants have been used in various traditional systems, as they immune potential against numerous diseases (Kottai Muthu et al., 2005). Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. More than 30% of the entire plant species, at one time or other was used for medicinal purposes. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment. Medical plants play an important role in the management of diseases in developing countries where resources are meager. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness (Balakumar et al., 2011, Rajan et al., 2011). The most important of these biologically active constituents of plants are alkaloids, flavonoids, tannins and phenolic compounds. *Cissus quadrangularis* belongs to the taxonomic group Magnoliopsida and family Vitaceae. *Cissus quadrangularis* is an ancient medicinal plant

native to the hotter parts of Ceylon and India. It has been used by common folk in India for promoting the fracture healing process. It was prescribed in the ancient Ayurvedic texts as a general tonic and analgesic, with specific bone fracture healing properties. This review gives a brief idea about its botanical description, phytochemistry, Osteoblastogenesis activity and its molecular healing mechanisms in Osteoporosis (Baby Joseph et al. 2013).

Remediation of metal contaminates is an emerging field in the broad area of environmental bio-ago-technology. Plant based remedies have always been an integral part of traditional medicine throughout the world. The increasing demand for herbal medicines in recent years due to their fewer side effects in comparison to synthetic drugs and antibiotics has highlighted the need for conservation and propagation of medicinal plants. The health benefits of a plant based diet are immense. It can prevent, retard or even reverse many life threatening disease processes (Gurumoorthy, 2005).

Oreochromis mossambicus (Peters, 1852) displays many favourable attributes as culture species; on the basis of its general hardness, resistance to disease, high yield, potential and ability to grow on a wide range of natural and cheap artificial foods. Additionally it can also withstand low oxygen concentration, overcrowding, tolerates difficult ecological conditions and a wide range of salinities and still produces a highly acceptable flesh (El-Sayed, 2006). So, Tilapias are the second only to carps as the most widely farmed fresh water fish in world (FAO, 2010).

Histological analysis of the tissues is considered a good indicator of the nutritional status of fish. Several studies have reported the fish organ histology (Sakr and Gabr, 1991; Muniyan, 1999; Aruldoss et al. 2014; Aysel Şahan, 2017). Histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs such as the gills, muscle, liver and kidney (Dutta, 1996). Histological investigation may therefore prove to be a cost effective tool to determine the health of Organisms hence reflecting the health of an entire aquatic ecosystem (Johndevadoss and Ramanibai, 2014). However, histology is the most useful tool for determining action of the stimulator at tissue level as it provides useful data concerning tissue health or recovery.

MATERIALS AND METHODS

Procurement and rearing of experimental fishes.

Oreochromis mossambicus commonly called African Mouth breeder is widely distributed in the freshwater of India. Even though the fish O. mossambicus is a freshwater fish but they can able withstand estuarine environment. The fish Oreochromis mossambicus ranging in length 10-18 ± 1.5 cms in total length and 40-70 ± 6.25 grams in weight were collected using hand nets from Gadilam river, Devnampattinam village, Cuddalore, Tamil Nadu, India. The collected fishes without any least disturbance were transported in polythene bags filled half with water and were stocked up in 500 litre capacity tank having dechlorinated tap water. The fishes were acclimatized for 15 days. This mode of transit proved successful, since there was no mortality in all consignments throughout the course of this study. The fishes were fed twice daily with prepared fish feed pellets.

Collection of Cissus quadrangularis

The plant Cissus quadrangularis was freshly collected from the rural area nearer to Periyapet village, Cuddalore district sufficient quantity in a sterile polythene cover and transported to the laboratory. Then chopped to pieces and crushed to paste with required amount of water. The paste weighed to 200grams and packed in polythene cover stored in the refrigerator for further use.

Preparation of Fish Feed

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

Control feed - Feed 1

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 porri (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°C. Proximate analysis of diets was carried out using standard methods (AOAC, 1990).

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	1.100	2.200
(Riboflavinin micrograms)		

Plant extracts preparation

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 ratio for 24 hours on a shaker at 200 r.p.m. 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°C and used for further analyses.

Experimental Procedure

Oreochromis mossambicus weighing 40-55gms were divided into 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, 14 and 21 days. Group I Fish exposed to tap water and fed with feed -I (Control feed 1) 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) Group IV Fish exposed to tap water and fed with feed 2 (Cissus quadrangularis extract mixed for 21 days)

Experimental design

The experiment was design to elucidate the effect of the Cissus quadrangularis plant extract on the tissues (gills, liver and muscles) of fish Oreochromis mossambicus in their histological changes in normal and plant extract treated 7, 14 and 21 days of treatment. LC 50 values are not calculated because the plant extract is not a toxic material to living beings but promotes the physiological efficiency in various ways of the organisms. Cissus quadrangularis aqueous plant extracts for the fish Oreochromis mossambicus (batch of 10 fishes) after 96 hours in semi static system was 500ppm to see the histopathological changes in the gills, liver and muscles after the completion of 21 days. The fishes were active throughout the period of study.

The fishes brought to the laboratory were acclimatized in fibre fish tank 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 trough. The fishes were exposed to plant extract and control for the period of 7, 14 and 21 days. A control group was maintained with identical environment. The Cissus quadrangularis plant extract with water and normal water was renewed every day. The fish were sacrificed from both experimental and control groups on 7, 14 and 21 days of exposure periods. The fishes were active throughout the period of study

Histological Studies

To examine the extent of cellular changes the tissues of gills, liver and muscles of the control and treated tissues were fixed in Bouin's fluid. After 24 hours, the standard histological technique was followed by the method of Gurr (1959). The *Cissus quadrangularis* aqueous plant extracts added water was renewed after 24 hrs with the same concentration. One group of fish did not receive any plant extract and this group served as the control. After the completion of 21 days, the fishes from the exposure group and also the control group were dissected and the gill liver and muscle were carefully removed, washed in 0.9 % saline and were then fixed in 10% formalin for 24 hrs. The tissues were dehydrated in ascending grades of alcohol. After dehydration in graded series of alcohol, they were cleared in acetone and xylol. Then, they are treated with cold and hot infiltration and embedded in paraffin wax (58oC-60o C). Serial sections were cut at 6 thickness, deparaffinized in xylol, passed through descending grades of alcoholic series, hydrated in water, stained in Heidenhain's iron haematoxylin and counter stained with aqueous eosin. Stained sections were mounted in DPX for microscopic observations. Microphoto graphs were taken in the laboratory by using Magnus compound microscope attached with camera Olympus OM-D E-M10 model.

RESULTS

Oreochromis mossambicus was treated with the plant extract of *Cissus quadrangularis* to study about the changes in the histology aspects of tissues (gills, liver and muscles). Experiments and results were conducted and recorded respectively.

Histological changes in the Gills

Normal

Oreochromis mossambicus gill structure consists of highly vascular shield structures called primary and secondary lamellae. The primary gill lamellae (PGL) are compressed flat leaf like structure on either side of the interbranchial septum. The interbranchial septum is narrow and shows highly narrow inflexible structure. Each one of them bears a row of secondary gill lamellae (SGL) on both sides perpendicular to the long axis of primary gill lamellae. The secondary gill lamellae are highly vascularised and surrounded by a thin layer of epithelial cells. Between the two adjacent respiratory lamellae lie the interlamellar region (Fig. 1A & 2A)

Treated

The plant extract of *Cissus quadrangularis* exposed to the gill of *O. mossambicus* showed marked histological changes. Appreciable changes were noted in the histology of gill after 14 days treatment including wider space and indifference of epithelium from the interbranchial septum, fusion of secondary lamellae and vacuolization. Dialation was more severe and the gill epitheliums surrounding the axis of primary and secondary lamellae were healthy to a great extent. Hyperplasia of the epithelial cells and lifting of the lamellar epithelium were noticed (Fig. 1B & 2B).

Fig. 1 Sections of control (A) and plant extract treated fish gill for 21 days (B) fish gill showing the general structure, gill rakers, primary gill lamella (PGL), secondary gill lamella (SGL), filamental epithelium (FE), veinous sinus(VS)

Fig.A

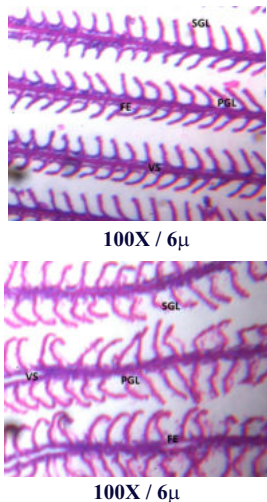
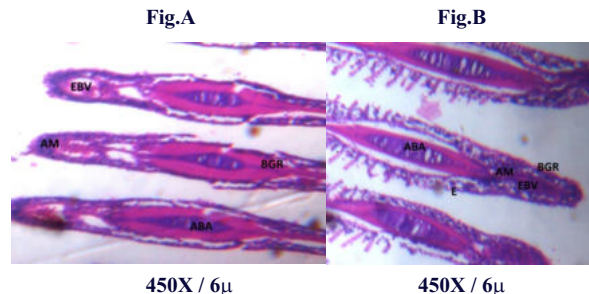


Fig.2 Sections of control (C) and plant extract treated fish gill for 21 days (D) fish gill showing the general structure basal part of gill rakers (BGR), filamental epithelium, abductor muscle(AM), veinous sinus, afferent branchial artery(ABA) and efferent branchial vein(EBV)



Histological changes in the Liver

Normal

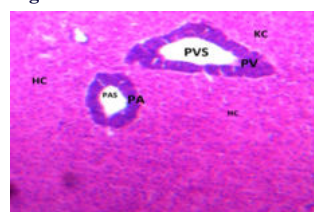
The normal sections of liver of *O.mossambicus* show the cords of hepatocytes tend to have a regular radial pattern enclosing the sinusoidal network for a short distance into the perivenular areas. These areas become less regular outside the perivenular zone. The individual hepatocyte is polygonal in shape and has a single spherical nucleus. The nuclei are mostly centrally located within the hepatocytes with some nuclei tending to occur closer to the cell periphery bordering the sinusoids. The endothelial cells that line these sinusoids as well as their nuclei are flattened and elongated. The portal artery and portal vein become very clear and portal artery sinus and portal vein sinus were very clear. Kuffer cells are without a remarkable boundary and are found in two or three here and there (Fig. 3A &3B).

Treated

Histological examination of *Cissus quadrangularis* plant extract treated fish *O.mossambicus* for 21 days revealed that healthy effect on the appearance of the liver structure and hepatocytes which had essentially slightly more than normal appearance. An improvement in the histological structure of the hepatocytes was noticed as compared to the control ones. The boundary between cells was noticed more or less clearly and the nuclei of the hepatic cells were seen to be enlarged and hyperchromatic (Fig. 3C & 3D).

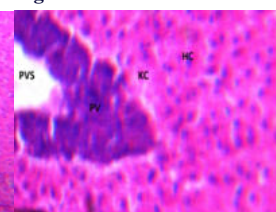
Fig.3 Sections of control (A) and plant extract treated for 21 days(B) fish liver showing the general structure, blood sinusoids (BS), portal vein (PV), portal vein sinus (PVS), portal artery (PA), portal artery sinus (PAS), hepatocytes (HC) and kuffer cell (KC)

Fig.A



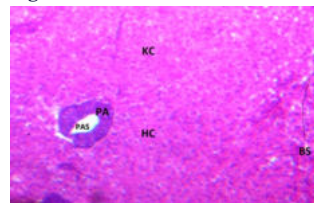
100X / 6µ

Fig.B



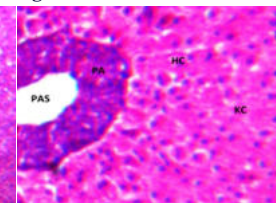
450X / 6µ

Fig.C



100X / 6µ

Fig.D



450X / 6µ

Histological changes in the Muscles

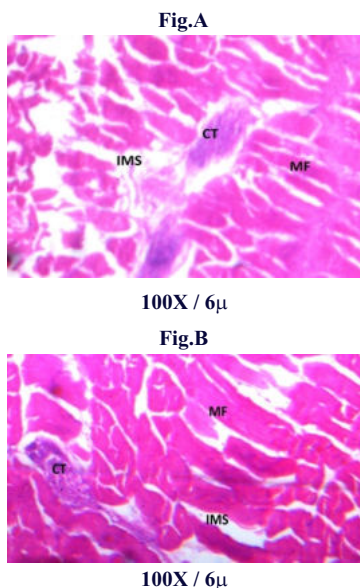
Normal

The normal muscle of *O.mossambicus* is made up of compact mass of myobrils arranged in regular interspace of endomysium. The muscle cells are in bundles and arranged at regular intervals with distinct nuclei. Large number blood sinusoids were also seen around the muscle bundles (Fig. 4A)

2 Treated

The plant extract of *Cissus quadrangularis*, obvious effects were observed at the structural and cellular level in the muscles. The main modifications found in the musculature were more endomyocytic space distortion in the myofibrils. In 7 days of exposed fish, disintegration of cell boundaries of the muscles and slight condensation of blood sinusoids were observed after 14 days, many adipocytes cells were observed and intracellular vacuolation was also apparent. Degeneration of contractile fibrils showing distinct vacuolated space and necrosis were the maximum alterations observed (Fig. 4B).

Fig. 4 Sections of control (A) and plant extract treated for 21 days (B) fish muscle showing the general structure, muscle fibers, fibril layers and connective tissue (CT) covers the muscle fibres, myofibrils, myofilaments (MF) and intermyofibrillar space (IMS)



DISCUSSION

Oreochromis mossambicus Peters, commonly called as tilapia is a salt-tolerant, mouth brooding cichlid native to Africa having immense aquaculture potential. *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. In the present study, the experiment was designed to elucidate the effect of the *Cissus quadrangularis* plant extract on the tissues (gills, liver and muscles) of fish *Oreochromis mossambicus* in the histological changes in normal and 7, 14 and 21 days of treatment.

Histological studies

The gills being delicate structures get affected easily if the surrounding media is contaminated (Roy and Dutta; Munshi, 1991). Palikova et al., (2004) have reported the protective effect of cyanobacteria extract on histopathology of carp. Mohamed et al., (2003) have suggested that the metabolic and histological studies on the effect of garlic administration on the carnivorous fish *Chrysichthys auratus*.

In the present study histologically pronounced regeneration of gill tissues resulting wider interbranchial septum were found in plant extract exposed fish fed with feed 2 (21 days). It is suggested that the hyperplasia of epithelial cells in the gill lamellae may be due to the pharmacological activity of *Cissus quadrangularis*. Histological analysis of liver was carried out in this study as it plays an important part in many vital functions of the basic metabolism and is also the largest organ responsible for accumulation, biotransformation and contaminant excretion, including pollutant degradation and bioactivation (Matos et al. 2007). Herbal medicines have recently gained much attention as alternative medicine to treat or prevent human diseases. Many active plant extracts are frequently utilized to treat a wide variety of clinical diseases including liver disease (Chattopadhyay, 2003). In the present study histology of the *Cissus quadrangularis* extract exposed liver shows enhanced activity of kuffer cells. The hepatocytes are polygonal with enlarged nucleus in the plant extract treated liver rather than the normal liver of *Oreochromis mossambicus*. Similar findings have been reported by Blair et al (1990); Shivasri, (2013). *Cissus quadrangularis* plant extracts have been suggested that are having more hepatoprotective activity.

In muscle constituents a major portion of the body of fish, the muscular tissue performs “mechanical work” by contracting. The muscles, which are also sites of storage of fat present a similar picture (Natarajan and Balakrishnan Nair, 1976). In the present investigation, the improved histology of muscle fibre was well pronounced in *Cissus quadrangularis* extract exposed fish fed with feed 2 (21 days) when compared to control fish fed with feed 1 (normal) in addition to this presence of more connective tissues in the histology of muscles treated with the extract than the normal. These findings were confirmed with the earlier studies.

(Natarajan and Balakrishnan Nair, 1976). Dietary supplementation of cabbage and broccoli extracts to rats decreases free radical induced tissue damage (Fang et al., 1987). Dietary antioxidants and other nutrients play an important role in preventing cells from radical induced cytotoxicity (Citarasu (2010).

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