

# Antioxidant Activity of Ipomoea Aquatica on Sarcopenic Aged Male Albino Rats

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**ABSTRACT:** Sarcopenia is increasingly recognized as a biomarker of aging, characterized by an age related decline in skeletal muscle (SKM) mass, strength and quality that can lead to decreased mobility, increased risk of injury and decreased independence. Ipomoea aquatica is a green leafy vegetable, with rich source of vitamins, proteins and amino acids and many health benefits. Supplementation of crude powdered drug of I. aquatica, (50mg/100g body weight) suspended in saline was given bidose a day to the sarcopenic aged male albino rats. The treatment was given for 13 weeks. At the end 13 weeks the SKM tissues were collected and homogenized using appropriate buffer by Teflon pestle and then Biochemical parameters were analyzed. Levels of superoxide dismutase (SOD), catalase (CAT), GPx (Glutathione Peroxidase), Vitamin C (Vit., C), Vitamin E (Vit., E.), carnosine, during the supplementation of I. aquatica were found to increased significantly than untreated aged male albino rats. Enhancement of antioxidants supports to enhance the physiological antioxidant properties in the SKM. Carnosine is a potential antioxidant as defensive against degenerative diseases. The influence of I. aquatica on carnosine may be due to the enriched proteins and amino acids in the supplementary drug which would compensate the loss of amino acid involved in the synthesis of this antioxidant carnosine. This study suggests, the supplementation of I. aquatica to enhance the antioxidant activity through the increased availability of vitamins, flavonoids and carotenoids.

**KEYWORDS:** Ipomoea aquatica, Sarcopenia, Antioxidants, Carnosine, Vitamins.

## I. INTRODUCTION

Sarcopenia is the inevitable decline in muscle mass and function that occurs with age, leads to increased risk for disability (Da Silva Alexandre et al., 2014; Foulkner et al., 2007) and mortality, (Kim et al., 2014; Landi et al., 2012; Roubenoff and Castaneda, 2001; Fulle et al., 2004) and also negatively affects energy intake, nutrition, and respiratory function (Jonathan et al., 2001). The age-associated loss of skeletal muscle mass and strength (Sarcopenia) is an unavoidable part of aging (Lee et al., 2007). Sarcopenia is likely mediated, at least in part, by a lifetime of damage from oxidants. Aging is associated with an increase in oxidant production and a decrease in the capacity to buffer oxidants, resulting in a chronic state of oxidative stress, which can damage biomolecules (DNA, lipids and proteins), decrease muscle protein synthesis elevate apoptotic signaling and protein degradation (Ryan et al., 2010). The previous research study suggest, Sarcopenic gastrocnemius muscle of young and older rats revealed increased reactive oxygen species (ROS), proteolysis, overproduction and vulnerability to apoptosis (Martin et al., 2007). The reactive oxygen species (ROS) are involved not only in muscle damage but are also able to modulate skeletal muscle contraction (Stefania Fulle et al., 2004). Older peoples may benefit from physical activity through the exercise-induced adaptation in cellular antioxidant defense and mitochondrial biogenesis. Strenuous exercise can increase muscle oxygen flux and elicit intra cellular events that may lead to increased oxidative damage to mitochondria (Ji, 2001). Intramuscular mitochondrial DNA damage caused by free radicals may be a significant factor in the loss of muscle mass seen in the aged (Ashok and Ali, 1999).

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## ANTIOXIDANT

Antioxidant is a substance that scavenging or deactivating the free radicals, suppressing their formation, or opposing their actions before them attack cells (Khalid Rahman, 2007). Humans have evolved highly complex antioxidant systems such as enzymatic and non-enzymatic antioxidants, which work synergistically, and in combination with each other to protect the cells and organ systems of the body against free radical damage (Mates et al., 1999). The antioxidants can be endogenous or obtained exogenously eg, as a part of a diet or as dietary supplements. Some dietary compounds that do not neutralize free radicals, but enhance endogenous activity may also be classified as antioxidants (Khalid Rahman, 2007).

### ENZYMATIC ANTIOXIDANTS

Endogenous antioxidants play a crucial role in maintaining optimal cellular functions and thus systemic health and wealth being. Cells are equipped with different kinds of mechanisms to fight against ROS and to maintain the redox homeostasis (Chanda and Dave 2009). However, under conditions which promote oxidative stress, endogenous antioxidants may not be sufficient and dietary antioxidants may be required to maintain optimal cellular functions. (Khalid Rahman, 2007).

#### SUPEROXIDE DISMUTASE (SOD) (EC NO:1.15.1.1)

Superoxide dismutase is one of the most effective intracellular enzymatic antioxidants and it present in all major aerobic tissues, which can scavenging the free radicals and protect from cell injury (Bergendi et al., 1999). SOD exists in several iso-forms, which differ in the nature of active metal centre, amino acid composition, co-factors and other features. There are three forms of SOD present in humans: cytosolic Cu, Zn-SOD, mitochondrial Mn-SOD, and extra cellular-SOD (Landis and Tower 2005). The function of the enzyme seems to be that of protecting aerobic organisms against the potential deleterious effects of superoxide anion. SOD catalyzes the conversion of superoxide anion to dioxygen and hydrogen peroxide (Chaudiere and Ferrari-Iliou, 1999).

#### CATALASE (CAT) (EC NO:1.11.1.6)

Catalase is an important enzymatic antioxidant, play important roles in scavenging the free radicals and preventing cell injury (Bergendi et al., 1999). Catalase present in the peroxisome of aerobic cells and very effective in promoting the conversion of hydrogen peroxide to water and molecular oxygen, it has one of the highest turnover rats for all types of enzyme (Mates et al., 1999).

#### GLUTATHIONE PEROXIDASE (GPX) (EC NO: 1.11.1.19)

Glutathione peroxidase acts in association with GSH, which is present in high concentration in cells and catalyzes the conversion of hydrogen peroxide to water or alcohol while simultaneously oxidizing GSH (Khalid Rahman, 2007; Bergendi et al., 1999)). It also competes with catalase for hydrogen peroxide as a substrate and is the major source of protection against low levels of oxidative stress (Chaudiere and Ferrari-Iliou, 1999).

### NON-ENZYMATIC ANTIOXIDANTS

Some dietary compounds that enhance endogenous activity of enzymes may also known as nonenzymatic antioxidants (Khalid Rahman, 2007). The most efficient nonenzymatic antioxidants such as Vit., C, Vit., E, carnosine and another compounds are neutralise and or prevent the cell damage from the free radical attack (Mc Call and Frei, 1999).

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## VITAMIN C (VIT., C)

Vitamin C is an important powerful water-soluble antioxidant and thus works in aqueous environment of the body, it found in the cytosol and extracellular fluid that can interact directly with free radicals, thus preventing oxidative damage (Ryan et al., 2010).

## VITAMIN E (VIT., E)

Vitamin E is a fat soluble vitamin existing in eight different forms. In humans,  $\alpha$  tocopherol is the most active form, and is the major powerful membrane bound antioxidant employed by the cell (Hensley et al., 2004). The main function of vit., E is to protect against lipid peroxidation chain reactions in cellular membranes by interfering with the propagation of lipid radicals (Pryor, 2000; Ryan et al., 2010). Vit., E may help to prevent sarcopenia in the elderly (Semba et al., 2003).

## CARNOSINE

Carnosine is a potential antioxidant as defensive against degenerative diseases. The marked reduction in muscle carnosine concentration may because of the age related decline in muscle mass, strength and function (Stuenkel and Kunne, 1999). In addition, recent data suggest that antioxidant supplementation can stimulate muscle protein synthesis in aged rats (Ryan et al., 2010).

Carotenoids are mainly colored pigments present in plants and microorganisms and epidemiological studies have revealed that an increased consumption of a diet rich in carotenoids is correlated with a lower risk of age-related diseases. Carotenoids contain conjugated double bonds and their antioxidant activity arises due to the ability of these to delocalize unpaired electrons (Mortensen et al 2001). This is also responsible for the ability of carotenoids to physically quench singlet oxygen without degradation and for the chemical reactivity of carotenoids with free radicals (Stahl and Sies, 2003).

The leafy vegetable *I. aquatica* commonly known as water spinach belongs to the family Convolvulaceae. It is a tender, trailing or floating perennial aquatic plant, found on moist soil along the margins of fresh water, marshes, and wet rice field. This study investigates the regulation of antioxidant activity on sarcopenia during the supplement of crude *I. aquatica* herbal drug.

## II. MATERIALS AND METHODS

### ANIMALS AND PLANT COLLECTION

Aged male albino rats were purchased and maintained under standard experimental conditions (temperature  $27 \pm 2^\circ\text{C}$ , relative humidity  $60 \pm 5\%$  12 hours light / dark cycle) and the weight of animal between 300 – 350g approximately. The average life span of male rats in this strain is ~31 months on an ad libitum diet (Fernandes et al., 1997). Weighed amount of pelleted balanced diet (Kamadhenu Agencies, Bangalore) and had free access to tap water ad libitum (100g of balanced diet were given per day). Food intake was assessed. Institutional Animals Ethics Committee (IAEC) had given the guidelines for maintenance of animals. In the experiment totally 2 groups of aged male albino rats were used. Each group contains 6 rats, group-I had normal untreated aged male albino rats, group-II had *I. aquatica* supplemented sarcopenic aged male albino rats. The herbal drug *I. aquatica* were collected from the area Manavayal near at Pattukkottai, Thanjavur district, Tamil Nadu, India. The shade dried and powdered leaves were used as drug. The crude herbal was suspended in saline (500mg / Kg body weight), crude herbal was given bidose a day to the aged male albino rats for 13 weeks. The treated rats were killed and the organs were collected. The weighed organs homogenised using appropriate buffer by Teflon pestle.

### PREPARATION OF SKELETAL MUSCLE HOMOGENATE

Hind leg muscles were freed of excess fat and connective tissues, finely mixed and washed in the medium containing 50mM TRIS buffer (pH 7.5), 100mM KCl, 5mM MgCl<sub>2</sub>, 1mM EDTA, 0.1% (w/v) fatty acid free bovine

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serum albumin (BSA). Tissue fragment was homogenized with the above medium (1:8, w/v) in a Potter Elvehjem homogenizer (Heidolph, Kelheim, Germany) set at 600 rpm and filtered through sterile gauze. Aliquots of the homogenate were then used for the Biochemical analysis.

## BIOCHEMICAL ANALYSIS

Homogenized organs were separately taken for the analysis. In the present study, the crude herbal drug of *I. aquatica* was investigated for their antioxidant activity on sarcopenic aged male rats. In vivo enzymatic antioxidant activity of superoxide dismutase (SOD) levels were assayed by using the method of Kakkar et al., (1984), catalase (CAT) levels were analysed by using Sinha (1972) method, GPx levels were estimated by using Rotruck et al., (1973) method, and the nonenzymatic antioxidant activity of Vitamin C levels were determined by the method of Omaye et al., (1979), Vitamin E levels were analysed by the method of Baker et al., (1980) and carnosine levels were analyzed by the method of Clifford, (1921) and Hunter (1922). All of these above mentioned estimations were carried out in the department of Siddha medicine laboratory, Tamil University. The results of *I. aquatica* crude herbal drugs in the activities of antioxidant enzymes are as illustrated on the table.

## STATISTICAL ANALYSIS

Results of each value are expressed as mean  $\pm$  standard deviation. Turkey Kramer Multiple comparison test was used to compare groups for Biochemical analysis, which were compared between groups by the use of an analysis of convariance using antioxidant activity estimation us a convariable. Values of  $p^* < 0.05$  were considered statistically significant.

## III. RESULT

Biological systems have protected against the harmful effects of free radicals, by the activities of antioxidants and its enzymes such as SOD and CAT (Zhao et al., 2011), which were found, the antioxidant activity of SOD ( $0.228 \pm 0.021$ ), CAT ( $0.628 \pm 0.05$ ) and GPx ( $0.721 \pm 0.054$ ) were significantly increased ( $p^{***} < 0.001$ ) in SKM tissues of *I. aquatica* supplemented aged male rats, when compared with untreated aged male control rats. Oral administration with *I. aquatica* showed highly significant effect and maintained the levels to near normal.

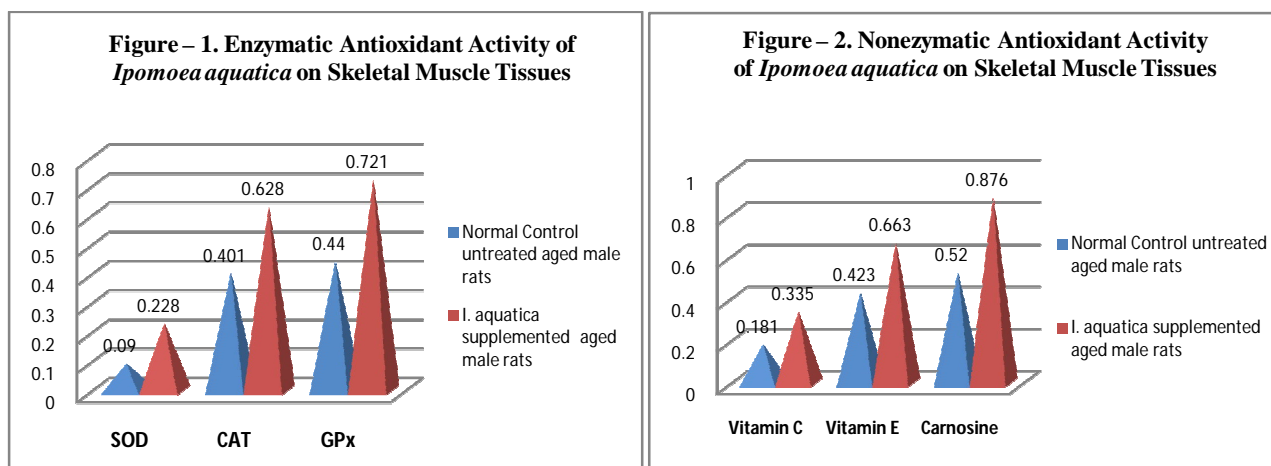
The increased level of Vitamin C ( $0.335 \pm 0.027$ ), Vitamin E ( $0.663 \pm 0.03$ ), and Carnosine ( $0.876 \pm 0.064$ ) content in skeletal muscle tissues of *I. aquatica* supplemented aged male rats compared with untreated aged male control rats. Supplementation of *I. aquatica* had restored the normal levels of carnosine vitamin C and vitamin E. This treatment also elicited therapeutic efficacy in altering this non-enzymatic antioxidant.

The concentration of physiological antioxidant carnosine in the skeletal muscle was found to increase in the treated rats than control. Administration of *I. aquatica* had influenced the level of carnosine in the muscular tissue.

**TABLE – 1. ANTIOXIDANT ACTIVITY OF I. AQUATICA ON SKELETAL MUSCLE TISSUES**

Variable	Normal Control untreated aged male rats	<i>I. aquatica</i> supplemented aged male rats
<b>Enzymatic Antioxidants</b>		
SOD (50% of NBT reduction/min/mg protein)	$0.09 \pm 0.014$	$0.228 \pm 0.021^{***}$
CAT ( $\mu$ mol H <sub>2</sub> O <sub>2</sub> consumed/mg Protein)	$0.401 \pm 0.019$	$0.628 \pm 0.05^{***}$
GPx (m.mol GSH utilised/min/mg Protein)	$0.44 \pm 0.021$	$0.721 \pm 0.054^{***}$
<b>Nonenzymatic Antioxidants</b>		
Vitamin C ( $\mu$ g/mg Protein)	$0.181 \pm 0.014$	$0.335 \pm 0.027^{***}$
Vitamin E ( $\mu$ g/mg Protein)	$0.423 \pm 0.03$	$0.663 \pm 0.03^{***}$
Carnosine ( $\mu$ mol/ mg Protein)	$0.52 \pm 0.021$	$0.876 \pm 0.064^{***}$

Data are expressed as mean  $\pm$  SD. Means among groups (n = 6 rats / group).  
Statistical significance:  $p^* < 0.05$ ,  $p^{**} < 0.01$ ,  $p^{***} < 0.001$ , Non-significant (N.S).



#### IV. DISCUSSION

The aim of this research study to analyse the efficacy of *I. aquatica* activity in both enzymatic and nonenzymatic level, and to compare *I. aquatica* supplemented sarcopenic aged male rats with untreated rats. The results revealed that the changes in the enzymatic antioxidant and non enzymatic antioxidant in skeletal muscle due to the bidose of sarcopenic aged male rats. Several studies have shown that the reduced level of antioxidant during the sarcopenic condition (Caroline Martin et al., 2006; Fulle et al, 2004; Wanagat et al., 2001). A major defensive mechanism involves the antioxidant Enzymes, including SOD, CAT and GPx, which convert active oxygen molecule into non-toxic compounds (Omale James et al., 2009). Some of these enzymes are also present in plants. In plants SOD isoenzymes are present in the cytosol and mitochondria (Bhathal et al., 1993; Omale James et al., 2009). Our finding on SOD activity showed significant increase in the *I. aquatica* supplemented SKM tissues of sarcopenic rats, which is in agreement with earlier reports (Caroline Martin et al., 2006).

The earlier report revealed that the CAT and GPx activity was increased during the supplementation of Vitamin E and C in the SKM tissues of aged rats and increase in basal levels of oxidative stress associated with aging, allowing for improved adaptation in oxidative enzymes and muscle function (Michael et al., 2010).

The maintenance of SOD, CAT and GPx during *I. aquatica* treatment signifies its therapeutic potential in eliminating the free radical accumulation. It is evidenced that vitamin C is the effective free radical quencher. The availability of vitamin C and flavonoids might have influenced, inhibited the free radical accumulation and thus influenced the endogenous antioxidant enzymes SOD and CAT. The presence of copper, zinc, manganese in *I. aquatica* might also evidence the structural integrity of enzyme and thus increase the functional activity. The presence of iron (Fe) in the drug would also have potentiated the CAT activity which is an integral element. The aqueous extract of *Cassia auriculata* flower which increase SOD, CAT and GPx were reported by Pari and Latha, (2002). Our finding suggest its antioxidant activity with would scavenge the free radical or prevent the glycation process.

The levels of non-enzymatic antioxidant there found restored to normal during *I. aquatica* treatment. GPx level correcting may be crucial in protecting against the sarcopenia and reduced disulphide linkage of protein and other cellular molecules involved in tissue damage (Shan et al., (1990). Sarcopenic complication may be counteracted by *I. aquatica* administration; preventing the change of both enzymatic and non-enzymatic antioxidant induced by oxidative stress. The beneficial effect of *I. aquatica* is evidenced through its antioxidant property. GPx activity was greater in exercised muscles of aged rats (Ryan et al., 2010) was evidenced and supports our result findings.

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Vitamin C is a major nonenzymatic antioxidant that essential for detoxification process, that scavenges the free radical in both blood and tissues. Many studies have shown that Vitamin C protects against cell death triggered by various stimuli and this protection is associated with its antioxidant property. Vitamin C supplementation studies have shown a reduction in marks of oxidative DNA, lipid and protein damage, and in support, Vitamin C has been shown to regulate factors that can influence gene expression, apoptosis, and other cellular functions (You et al., 2000) The elevation of vitamin C during the supplementation of *I. aquatica* signifies it enriched vitamin C availability. Maintenance of vitamin C and vitamin E during therapeutic treatment with *I. aquatica* signifies the recycling of oxidized ascorbic acid (vitamin C) and oxytocopherol (vitamin E) due to accumulated free radical. *I. aquatica* have been demonstrated to have antioxidant property which would have directly scavenged free radicals and thus increased the availability of antioxidant (Sen et al., 1992; Sen et al., 1994) vitamin E act as chain breakers to stop the propagation sequential free radical reactions as can water soluble antioxidant such as vitamin C (Traber et al., 1999). The presence of vitamin E in the drug would also act as a co-antioxidant by regenerating  $\alpha$ -tocopherol from the tocopheroxyl radical during scavenging of ROS (Kharb. 2000). Treatment with *I. aquatica* to improve antioxidant activity in the sarcopenic muscle, and also prevented the pathogenic pro-oxidant by vitamin C, vitamin E and also flavonoids.

Carnosine is a potential antioxidant as defensive against degenerative diseases. Previous study reports that the marked reduction in muscle carnosine concentration may be related decline in muscle mass strength and function (Stuenrenburg and Kunne, 1999). The influence of *I. aquatica* on carnosine may be due to the enriched proteins and amino acids in the supplementary diet which would compensate the loss of amino acid involved in the synthesis of carnosine. Availability of  $\beta$ -carotene, vitamin C in this dietary vegetable might have been implicated to enhance this physiological antioxidant in the skeletal muscle, thus increasing the level of cellular antioxidant defenses. The recent studies confirm that increase in muscle carnosine concentration, lead to increased intracellular hydrogen ion ( $H^+$ ) buffering capacity (Dunnet and Harris, 1999).

## V. SUMMARY AND CONCLUSION

Health promotion in the elderly has been a major recent public health concern. Sarcopenia is a term utilized to define the loss of muscle mass and strength that occurs with aging, is widely considered one of the major causes of muscle weakness, increased risk of injury and disability in older persons with aging there is a decline in food intake, this occurs to a greater extent in men than women. The magnitude of the public health problem posed by sarcopenia is not yet established. The purpose of this research study is investigated sarcopenia and its relationship with functional status in elderly. Plant and its products are very low or no side effects, so the modern system of medicine go for the herbal medicine. The aquatic plant, *I. aquatica* was a green leafy vegetable, these have antioxidant property. Therapeutic treatment with *I. aquatica* to sarcopenic aged male rats had found to increase the endogenous antioxidant enzymes SOD, CAT and GPx which lowered free radical in sarcopenic muscles.

Further the non-enzymatic antioxidants such vitamin C, vitamin E and carnosine were found enhanced during drug treatment is skeletal muscle which is found increased during *I. aquatica* supplementation, suggesting the adaptive response against oxidative stress during sarcopenic condition. The endogenous antioxidant peptide carnosine was found decreased in the skeletal muscle in untreated aged male sarcopenic rats. Administration of *I. aquatica* had increased the carnosine content in the skeletal muscle.

Therapeutic efficacy of *I. aquatica* have enriched antioxidant like SOD, CAT, GPx, vitamin C, vitamin E and caronsine, which scavenge the free radical and it prevent from the oxidative damage to the skeletal muscle tissues. The results of the present study suggest the beneficial effect of *I. aquatia*, as a natural dietary supplement with enriched vitamins, minerals, antioxidant and phytonutrients.

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