

# ***Invivo* Antiinflammatory Activity of *Ipomoea Aquatica* on Sarcopenic Aged Male Albino Rats**

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**ABSTRACT:** The elderly people are prone to suffer from sarcopenia may be related to multiple factors, such as abnormal protein metabolism, oxidative stress, and chronic inflammations. Reactive radical compounds such as nitric oxide (NO), nitrogen dioxide (NO<sub>2</sub>), and non-radical compounds. NO, Glutathione-S-transferases (GST) and Myeloperoxidase (MPO) have been implicated in the loss of SKM mass and function that occurs during aging. NO and superoxide are generated by SKM and where these are generated in proximity their chemical reaction to form peroxynitrite can compete with the superoxide dismutation to H<sub>2</sub>O<sub>2</sub>. The primary ROS generated by skeletal muscle during contractions are superoxide and nitric oxide with rapid dismutation of the superoxide to hydrogen peroxide. Under physiological conditions, ROS are continuously produced in small quantities in the body. Nevertheless, this basal production is effectively controlled by the antioxidant defenses. In this case, there is equilibrium in the "oxidants-antioxidants" balance. However, if antioxidant defenses are no longer able to support the production of ROS, an overproduction of ROS in the cell causing irreversible damage, leading to an impaired redox homeostasis and disrupting intracellular signaling pathways. This phenomenon is commonly called oxidative stress (*Sies H, Cadenas, 1985; Jones, 2006*) and occurs in skeletal muscle during aging and sarcopenia. Production of free radical can also influence muscle mass by positively affecting expression of myofibrillar proteins in muscle experiencing increased loading. The dietary supplementation of *I. aquatica* ability to quench free radicals and having anti-inflammatory activity. It is also evidenced that the plant had shown to inhibit NO which is due to the presence of polyphenolic compounds in this plant extract and also showed significant membrane stabilizing activity. Our study suggest that *I. aquatica* could be a useful natural leafy vegetable having rich source of vitamins, minerals, polyphenolic compounds, carotenoids, and flavanoids which comprises its utility as nutraceutical food and it can be used to cure inflammatory disorders.

**KEYWORDS:** *Ipomoea aquatica*, Sarcopenia, Antiinflammatory activity, NO, GST, MPO.

## **I. INTRODUCTION**

Aging is a process of deleterious and progressive changes in multiple organ systems, have important effects on body composition and health risk with time during post-maturational life and by a progressive inability to withstand stresses (*Cavallini et al., 2008*) which make the organism vulnerable to disease and increase the risk of death (*Masoro, 1999*). Loss of muscle mass is a natural phenomenon of aging, even great inter individual differences exist (*Melton et al., 2000*). All this leads to changes in body shape, where peripheral muscle mass decreases and fat stores tend to increase especially around the belly in men and around the hips in women (*Sieber, 2008*). A continuous loss of muscle mass can be observed in different populations and this loss of muscle mass is more pronounced in men than women. Normal aging is associated with a reduction in muscle mass and strength called **sarcopenia**. The consequences of the aging process in skeletal muscle are characterized by a decline in physiological function, reduced physical activity and

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a loss of muscle mass, quality and strength, a condition often referred to as sarcopenia (Kamel HK, 2003; Volpi et al., 2004; Powers et al., 2005). The elderly people are prone to suffer from sarcopenia may be related to multiple factors (Walrand, et al., 2011; Newman et al., 2003), such as abnormal protein metabolism, oxidative stress, and chronic inflammations. Reactive radical compounds such as nitric oxide (NO), nitrogen dioxide (NO<sub>2</sub>), and nonradical compounds, e.g., peroxynitrite (ONOO<sup>-</sup>) and dinitrogen trioxide (N<sub>2</sub>O<sub>3</sub>), are collectively called reactive nitrogen species (RNS). These free radicals are unstable because of the presence of unpaired electrons in their outer electron orbit. RNS is often linked to ROS, e.g., in the formation of peroxynitrite causing nitrosative stress. Oxidative and nitrosative stress have been etiologically implicated in a wide variety of disease processes and states: aging, hypertension, atherosclerosis, diabetic neuropathies, renal diseases, neurological diseases including Alzheimer's disease and other forms of dementia, as well as cancers (Butterfield et al., 2007; Forbes et al., 2008; Hamel et al., 2008). Oxidative stress also contributes to various gastro intestinal diseases including gastroduodenal ulcers (Peng et al., 2008), inflammatory bowel disease (Grisham, 1994; Pavlick et al., 2002), and gastro intestinal malignancies such as gastric (Kekec et al., 2009) and colorectal cancer (Inokuma et al., 2009).

Reactive oxygen and nitrogen species have been implicated in the loss of SKM mass and function that occurs during aging. Nitric Oxide (NO) and superoxide are generated by SKM and where these are generated in proximity their chemical reaction to form peroxynitrite can compete with the superoxide dismutation to H<sub>2</sub>O<sub>2</sub> (Pearson and Jackson, 2015). The primary ROS generated by skeletal muscle during contractions are superoxide and nitric oxide with rapid dismutation of the superoxide to hydrogen peroxide (Mc Ardle and Jackson, 2000). Under physiological conditions, ROS are continuously produced in small quantities in the body (free radical such as superoxide radical O<sub>2</sub><sup>-•</sup> or nitric oxide NO<sup>•</sup>; or non-radical species such as hydrogen peroxide H<sub>2</sub>O<sub>2</sub>). Nevertheless, this basal production is effectively controlled by the antioxidant defenses. In this case, there is equilibrium in the "oxidants-antioxidants" balance. However, if antioxidant defenses are no longer able to support the production of ROS, an overproduction of ROS in the cell causing irreversible damage, leading to an impaired redox homeostasis and disrupting intracellular signaling pathways. This phenomenon is commonly called oxidative stress (Sies H, Cadenas, 1985; Jones, 2006) and occurs in skeletal muscle during aging and sarcopenia (Sullivan-Gunn and Lewandowski, 2013; Andersson et al., 2011; Chabi et al., 2008; Jackson et al., 2011; Miller et al., 2012).

## Nitric Oxide

NO<sup>•</sup> is a weak oxidant, but when it combines with O<sub>2</sub><sup>-•</sup> to generate OONO<sup>-</sup>, it becomes a potent ROS (Koppenol, 1998). NO<sup>•</sup> and OONO<sup>-</sup> generate very stable nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) ions which accumulate in cells, leading to the formation of highly reactive intermediates, such as NO<sub>2</sub>, N<sub>2</sub>O<sub>3</sub>, or NO. These intermediates cause nitration and nitrosation of important biological macromolecules such as DNA, RNA, proteins, and lipids, thereby disrupting their function. 8-Nitroguanine, a nitration product of DNA and RNA, is a potent mutagen and pro-oxidant formed within the cells (Kaneko et al., 2008). Nitrated lipids are capable of eliciting varied physiological responses and can also produce diffusible NO (Bhattacharyya et al., 2014). Previous data have indicated that contractile activity increases NO release by skeletal muscle (Balon and Nadler, 1994; Pattwell et al., 2004). However, numerous additional cell types (e.g. endothelial cells, smooth muscle cells) may contribute to the NO released into interstitial fluid. NO released from these sources may therefore dilute the effect of any NO released from contracting skeletal muscle (Hirschfield et al., 2000).

## Glutathione-S-transferases (GST)

Glutathione-S-transferases (GST) are a group of enzymes that catalyse the conjugation of GSH to a variety of electrophiles, thereby decreasing its reactivity with cellular components (Bickers and Athar, 2006). There are four different Se-dependent glutathione peroxidases present in humans (Chaudière and Ferrari-Iliou 1999), and these are known to add two electrons to reduce peroxides by forming selenoles (Se-OH) and the antioxidant properties of these seleno-enzymes allow them to eliminate peroxides as potential substrates for the Fenton reaction. Selenium-dependent glutathione peroxidase acts in association with tripeptide glutathione (GSH), which is present in high concentrations in cells and catalyzes the conversion of hydrogen peroxide or organic peroxide to water or alcohol while simultaneously

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oxidizing GSH. It also competes with catalase for hydrogen peroxide as a substrate and is the major source of protection against low levels of oxidative stress (Chaudière and Ferrari-Iliou 1999).

## Myeloperoxidase (MPO)

Myeloperoxidase (donor: H<sub>2</sub>O<sub>2</sub> oxidoreductase, EC 1.11.1.7) appears to be uniquely localized in mammalian neutrophils and monocytes (Klebanoff and Clark, 1978). A major function of neutrophils is to kill ingested bacteria; a function partially fulfilled by myeloperoxidase. This enzyme has been shown to have a bactericidal effect in the presence of H<sub>2</sub>O<sub>2</sub> and a halide such as chloride, iodide, or bromide (Andrews and Krinsky, 1988). Myeloperoxidase (MPO) is a marker of oxidative stress and inflammation. It is tissue inhibitor of metalloproteinases, and total plasminogen activator inhibitor -1 (Toneto et al., 2016). MPO is a major end-product of NO metabolism, readily promotes tyrosine nitration through formation of nitryl chloride (NO<sub>2</sub>Cl) and nitroendioxide (NO<sub>2</sub>) by reaction with the inflammatory mediator MPO (Eiserich et al., 1998).

## II. MATERIALS AND METHODS

### Animals and Plant collection

Aged male albino rats were purchased and maintained under standard experimental conditions (temperature 27 ± 2°C, relative humidity 60 ± 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 serum and the organs were collected. The weighed organs homogenised using appropriate buffer by Teflon pestle. Serum used for the biochemical analysis.

### Preparation of Skeletal muscle and Liver homogenate

Hind leg muscles were freed of excess fat and connective tissues, and liver tissues were 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 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 dietary supplementation of *I. aquatica* was investigated for their anti-inflammatory activity on sarcopenic aged male rats. *In vivo* anti-inflammatory activity of Nitric Oxide (NO) levels were assayed by using the method of Levine et al. (1976). Glutathione -S- Transferase (GST) levels were analysed by using Habig et al (1974) method and Myeloperoxidase (MPO) levels were estimated by using Holland et al., 1974, Levine et al., (1976) method. All of these above mentioned estimations were carried out in the department of Siddha medicine laboratory, Tamil University. The results of *I. aquatica* dietary supplementation in the activities of anti-inflammatory activity are as illustrated on the table.

**1. Estimation of Nitric oxide (NO):** Nitric oxide (NO) was estimate by the method of Levine et al. (1976). To 0.5 ml of tissue homogenate, 0.1 ml of sulphosalicylic acid was added and vortexed well for 30 min. the samples were than

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centrifuged at 5000 rpm for 15 min. the protein free supernatant was used for the estimation of nitrite levels. To 200  $\mu$ l of the supernatant, 30 $\mu$ l of 10% NaOH was added, followed by 300  $\mu$ l of TRIS HCl buffer and mixed well. To this 530  $\mu$ l of Griess Reagent was added and incubated in the dark for 10-15 minutes and the absorbance was read at 540 nm against a Griess reagent blank. Sodium nitrites solution was used as the standard. The amount of nitrite present in the samples was estimated from the standard curves obtained.

**2. Assay of glutathione –S- transferase (EC.No:2.5.2.18):** GST was assayed by the method of *Habig et al (1974)*. The reaction mixture contained 1.0 ml of phosphate buffer, 0.01ml of CDNB, 0.1ml of sample and 0.7 ml of distilled water. This mixture was pre incubated at 37° C for 5 minutes, and then the reaction was started by the addition of 0.1ml of GSH. The change in the absorbance was read at 340 nm for 5 minutes, the reaction mixture without the enzyme used as the blank. The enzyme activity was expressed as  $\mu$ mol CDNB – GSH Conjugates/ min/mg protein.

**3. Assay of Myeloperoxidase:** Myeloperoxidase activity was assayed using a noncarcinogenic compound (*Holland et al., 1974*). The reaction was carried out at room temperature in 50 ml of sodium acetate buffer and 5 mM H<sub>2</sub>O<sub>2</sub>. The reaction, initiated by the addition of enzyme, was followed by measuring the rate of appearance of the oxidation product of TMB at 655 nm with a Cary 14 spectrophotometer. One unit of activity is defined as the amount of enzyme necessary to decompose 1/mol of H<sub>2</sub>O<sub>2</sub>/min.

## Isolation Procedures

Isolation of mito Blood CeZCs-Native myeloperoxidase was isolated from out-dated leukophoresis preparations, which provided on the order of 10<sup>7</sup> white blood cells/batch. These out-dated preparations were obtained within 4 days of donation, during which time they were stored at 4°C. Whole cells were obtained by sedimentation at 500 X g for 15 min. Contaminating red blood cells were removed from the cell pellets by the addition of distilled water for 2 min followed by the addition of 0.6 M NaCl to restore isotonicity (*Levine et al., 1976*). The whole cells were centrifuged for 15 min at 500 X g and the washing cycle was repeated until the pellet became off-white.

**Isolation of Native Myeloperoxidase-**The white blood cells were lysed by the addition of 0.5% CTAB (*Harrison et al., 1977*) and cell debris was removed by centrifugation for 15 min at 15,000 X g at 5 °C. All subsequent centrifugations were carried out under these conditions. The supernatant was treated with solid NH<sub>2</sub>SO<sub>4</sub> to yield a final concentration of 50% saturation. This solution was held at 4°C for 30 min and centrifuged to remove the precipitate. The resulting supernatant was treated with solid NH<sub>2</sub>SO<sub>4</sub> to increase the concentration to 65% saturation, and was incubated at 4°C for 30 min prior to centrifugation. This procedure, a modification of a method described by *Agner (Agner, 1958)*, resulted in a pellet containing the bulk of the myeloperoxidase activity. Myeloperoxidase precipitated by the addition of NH<sub>2</sub>SO<sub>4</sub> was redissolved in a buffer containing 50 mM Tris, pH 7.0, and 0.5% CTAB (column buffer), and chromatographed on Sephadex G-150, superfine grade. To prepare this column, Sephadex G-150 was swollen at room temperature for 3 days in column buffer. The column, 2.5 X 30 cm, was washed with several volumes of column buffer before use and was run at room temperature.

## 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 anti-inflammatory activity estimation us a convariable. Values of p\* < 0.05 were considered statistically significant.

## III. RESULTS

The result shows the level of NO in Serum, SKM and Liver tissues (Table-1). No significant changes were observed in the level of serum NO; the NO level significant decreased in SKM and Liver in *I. aquatica* supplemented aged male rats when compared to untreated control aged male rats. The decreased levels of NO in SKM were 43% and

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in Liver tissues were 29%, but in serum NO level was non-significant. The level of **MPO** shows in Table-2, in Serum of *I. aquatica* supplemented aged male rats and untreated control aged male rats. The level of serum MPO was found to be highly significantly decreased in *I. aquatica* supplemented aged male rats when compared to untreated control aged male rats. The reduced level of MPO in serum 43% when compared with control aged male rats.

Organs	Control	<i>Ipomoea aquatica</i> supplemented
Serum	0.08 ± 0.008	0.07 ± 0.014 <sup>NS</sup>
SKM	0.138 ± 0.027	0.078 ± 0.013***
Liver	0.126 ± 0.021	0.09 ± 0.014**

Table-1. Effect on *Ipomoea aquatica* in Nitric oxide (NO) in Serum, SKM and Liver.

Parameters	Control	<i>Ipomoea aquatica</i> supplemented
MPO	3.44 ± 0.52	1.95 ± 0.37***

Table-2. Effect on *Ipomoea aquatica* in Serum MPO

Organs	Control	<i>Ipomoea aquatica</i> supplemented
SKM	5.866 ± 0.07	7.39 ± 0.068***
Liver	6.20 ± 0.075	8.371 ± 0.039***

Table-3. Effect of *Ipomoea aquatica* on SKM and Liver GST levels in control and treated rats.

All Data are expressed as mean ± SD. Means among groups (n = 6 rats / group). **NO**: n mol/g tissue. **MPO**: mg/dl **GST**: Units/mg protein. Statistical significance: p\*\*<0.01, p\*\*\*<0.001, Non-significant (NS). (Turkey- Kramer Multiple comparison Test). \*As compared with Control rats.

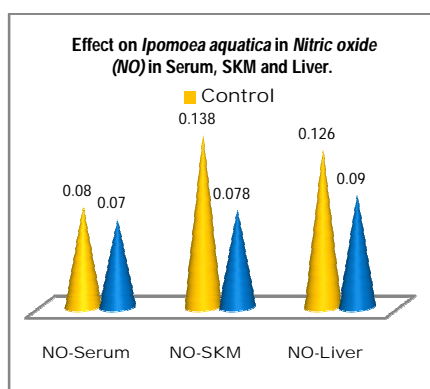


Figure-1.

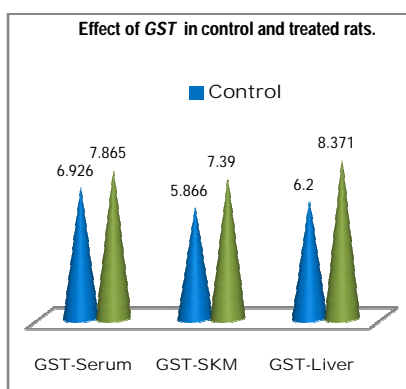


Figure-2.

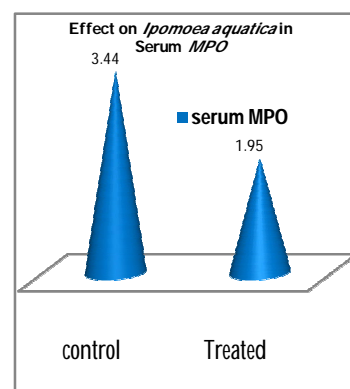


Figure-3.

Figure-1. Shows NO levels in Serum, SKM and Liver during *Ipomoea aquatica* supplementation.

Figure-2. Shows GST levels in Serum, SKM and Liver during *Ipomoea aquatica* supplementation.

Figure-3. Shows MPO levels in serum during *Ipomoea aquatica* supplementation.

The level of **GST** shows in SKM and Liver tissues of *I. aquatica* supplemented aged male rats and untreated control aged male rats. The level of GST in SKM and Liver was found significant increased in *I. aquatica* supplemented aged male rats when compared to untreated control aged male rats. The increased level of GST in SKM 25%, in Liver 35% when compared with control aged male rats.



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## IV. DISCUSSION

NO is a reactive nitrogen molecule that is formed enzymatically by nitric oxide synthases (NOS), via the conversion of L-arginine to L-citrulline. Skeletal muscle expresses three isoforms of NOS, namely the neuronal (nNOS), the endothelial (eNOS), and the inducible (iNOS) (Stamler and Meissner, 2001). Under normal physiological conditions both eNOS and nNOS synthesize NO at low levels, whereas iNOS expression is upregulated during inflammation and results in high levels of NO production (Stamler and Meissner, 2001).

Under certain conditions, NO reacts with superoxide anions ( $O_2^-$ ) to form the toxic molecule peroxynitrite (ONOO<sup>-</sup>), leading to oxidative stress and muscle fiber loss (Di Marco et al., 2005; Matata et al., 2002). Although the detailed mechanism of how NO<sup>-</sup> induced stress leads to muscle wasting remains to be elucidated, the production of NO, and the subsequent formation of peroxynitrite, has been shown to decrease mRNA levels of Myo-D an important transcription factor involved in myogenesis and maintenance of skeletal muscle (Di Marco et al., 2005; Guttridge et al., 2000). Furthermore, NO generated in response to both endotoxin and IFN- $\gamma$  has been found to inhibit general translation, leading to decreased protein synthesis in muscle fibers (Frost et al., 2009).

The role of nitric oxide synthase (NOS) induced oxidative stress in both sarcopenic and cachectic muscle wasting. Indeed, several studies have found an increase in the levels of protein nitration with age, suggesting an elevated level of NO and peroxynitrite production (Kanski et al., 2005; Viner et al., 1996; Haynes et al., 2010; Beal, 2002). An *in vivo* study evaluating the levels of NOS expression in old and young mice found a marked increase in the levels of NOS protein in older mice when compared to their younger counterparts (Hall et al., 2011).

Previous studies showed that NO can inhibit calpain activity, leading nNOS expression changed in aged muscle (Giuseppina Samengo et al., 2012). nNOS expression in skeletal muscle declined dramatically as muscle aged and that calpain-mediated proteolysis contributed significantly to sarcopenia and because NO is a negative regulator of calpain activity (Giuseppina Samengo et al., 2012), NO production by muscle increases during muscle disuse or following denervation (Suzuki et al., 2007), despite reductions in nNOS concentration (Nguyen and Tidball, 2003; Suzuki et al., 2007). Perhaps, the elevation in NO production when nNOS concentration is reduced reflects the higher cytosolic calcium concentrations in disuse atrophied muscles (Ingalls et al., 1999).

NO promotes atrophy by driving the expression of proteins associated with atrophy rather than inhibiting atrophy through calpain S-nitrosylation. Conversely, NO production can also influence muscle mass by positively affecting expression of myofibrillar proteins in muscle experiencing increased loading (Giuseppina Samengo et al., 2012).

Our present study suggest the NO level was found non-significant in serum but significantly decreased in SKM and Liver tissues during the supplementation of *I. aquatica* in aged male rats when compared with untreated control aged male rats. Our finding may suggest the dietary supplementation of *I. aquatica* ability to quench free radicals and having anti-inflammatory activity. It is also evidenced that the plant had shown to inhibit NO which is due to the presence of polyphenolic compounds in this plant extract and also showed significant membrane stabilizing activity (Omale et al., 2009).

The role of GST, catalyzing the reaction of a wide variety of electrophiles (chemical carcinogen) to GSH has been well established and formation of GSH conjugation of xenobiotics has been associated with the cellular detoxification system (Hartman and Shankel, 1990). In addition GST can bind covalently with a variety of carcinogen so the enhanced level of GST activity suggests further metabolic disposition of chemical carcinogens (Crrenius et al., 1984).

There are convincing evidences to support induction of GST and protection against a wide spectrum of cytotoxic, mutagenic and carcinogenic chemicals. Induction of GST activity in Luteolin pretreated groups support the detoxification capacity of the compound (Ketterer, 1998). The ethanolic extract of herbal drug increased the activity of

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hepatic GST in sodium arsenate-induced oxidative stress in rats. The earlier report revealed that the significant increase of liver GST activity in rats (Lima et al., 2005).

Our study suggests that increased activity of GST during the supplementation of *I. aquatica* may be an effective anti-inflammatory agent in SKM and Liver tissues, where in Phase II detoxification enzymes (GST) levels are induced. It is evidenced coordinately regulated detoxification enzyme system such as GST resistant to drug which can be related to effective anticancer drug (Schuetz et al., 1990). Previous study postulates the pre-treatment of rats with herbal drug could increase the activities of glutathione S-transferase in liver tissues (Lee et al., 2007).

It is evidenced earlier that resistance training can effectively increase the muscle content of limbs and reduce the level of inflammatory factors. In humans, low GST activity is associated with high tumor incidence, and vice versa. Glutathione/GST causes neoplastic changes in *H. pylori*-infected gastric mucosa (Verhulst et al., 2000). GST activity is reduced in colon cancer. Again, dietary intake of fruits and vegetables reduces the risk of colorectal cancer (Grubben et al., 2001; Bhattacharyya et al., 2014), which may in part be attributable to their ability to favor an antioxidant environment.

MPO is the major constituent of neutrophils. Neutrophils, which can export MPO into the extracellular environment where it may participate in a variety of biological effects. During NO metabolism MPO level was elevated and readily promotes tyrosine nitration through formation of  $\text{NO}_2\text{Cl}$  and  $\cdot\text{NO}_2$  by presence of MPO and it enhance inflammatory reactions (Eiserich et al., 1998). Previous report revealed that the MPO can participate in bacterial iodination mediated toxicity to mammalian cells, which is appear during the inflammatory disorders (Klebanoff and Hamon, 1972). Supplementation of *I. aquatica* could reduce the inflammatory process within the cell and ultimately reduce the MPO level in serum when compared with aged untreated control rats.

## V. SUMMARY AND CONCLUSION

Our finding may suggest the dietary supplementation of *I. aquatica* ability to quench free radicals and having anti-inflammatory activity. It is also evidenced that the plant had shown to inhibit NO which is due to the presence of polyphenolic compounds in this plant extract and also showed significant membrane stabilizing activity. The increased activity of GST during the supplementation of *I. aquatica* may be an effective anti-inflammatory agent in SKM and Liver tissues, where in Phase II detoxification enzymes (GST) levels are induced. It is evidenced coordinately regulated detoxification enzyme system such as GST resistant to drug which can be related to effective anticancer drug. Supplementation of *I. aquatica* could reduce the inflammatory process within the cell and ultimately reduce the MPO level in serum when compared with aged untreated control rats. Our study suggest that *I. aquatica* could be a useful natural leafy vegetable having rich source of vitamins, minerals, polyphenolic compounds, carotenoids, and flavanoids which comprises its utility as nutraceutical food and it can be used to cure inflammatory disorders.

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