Defensive role of *Nardostachys jatamansi* Extract against Nicotine induced Toxicity on Skeletal muscle ATPase (Mg^{2+} and Ca^{2+} ATPases) and Creatine phosphokinase in Male Albino rat with reference to Aging.

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ABSTRACT

Nardostachys jatamansi (family Valerianaceae), an indigenous medicinal plant. It helps to promote physical and mental health augment resistance of the body against disease and has shown potent antioxidant activity. Nicotine is known to induced oxidative stress and depletes antioxidant defense mechanisms in skeletal muscle tissue, on the basis of a antioxidant properties N.Jatamansi have been chosen for the present study. Pathogen free, Male Wistar strain albino rats (3 months as a Young, 18 months as a Old) were used in the present study, rats were divided into 4 groups of six rats in each group and treated as follows :Group I: Normal Control; Group II: Nicotine treated (Nt) (at a dose of 0.6 mg/ kg body weight by subcutaneous injection for a period of 2 months);Group III: Nardostachys Jatamansi Extract treated (NJEt) (50mg/kg body weight) via Orogastric tube for a period of 2 months); IV: Nicotine + Nardostachys Jatamansi Extract treated (Nt+NJEt), rats were received the nicotine at a dose as mentioned in Group II through subcutaneous injection and N. Jatamansi Extract as mentioned in Group III via Orogastric tube for a period of 2 months.The animals were sacrificed after 24 hrs after the last treatment session by cervical dislocation. In the present investigation reveals that all ATPase'(Mg^{2+} and Ca^{2+}) activities and Creatine phosphokinase (CPK) were decreased in nicotine treated rats and enhanced was observed in NJEt rats over the control. In the combination treatment (Nt+NJEt) up regulation was observed. This review highlights the importance medicinal properties on skeletal muscle tissue and function and other related disorders, in particular their mechanism of action and therapeutic potential.

Key words: Nicotine, Nardostachys Jatamansi Extract, ATPase (Mg^{2+} and Ca^{2+}),Creatine phosphokinase(CPK), Skeletal muscle Tissue and Male albino rat

INTRODUCTION

Nardostachys jatamansi Commonly known as Indian Nard or Spike nard or Nardus root, is an indigenous Indian drug which has been prescribed in this country since 800 B.C. The plant grows wild In the Himalayas at an altitude of 3,700-5, 0 00 m extending east-west from Kumaon to Sikkim and at 5,700 m in Bhutan. *N. Jatamansi* is a perennial herb containing a cylindrical rhizome covered with brown to deep greyish fibres.(Fig.2) There are number of compounds reported, which have been isolated from its roots and rhizomes, e.g. Sesquiterpene ketone - jatamansone (Govindachari *et al*., 1959), liquid alcohols - nardol, calarenol and n-hexacosanol; a ketone valeranone and diethenoid ketone - nardostachone, n-hexacosen, n-hexacosanyl isovalerate, n-hexacosanyl arachiedate, isovaleric acid, valeranal and p-sitosterol (Sastry et al.,

1967). seychelane, seychellene, valeranal, valeranone. Volatile essential oil, resins, sugar, starch, bitter extractive matter, gum, ketone, sesqueterpin ketone, spirojatamol etc. (Chatterjee, B.;**2005**; Bose, B.C *et al.*, **1957)** have been reported from powdered roots. Other sesquiterpenes include nardin, nardal, jatamnsic acid, b-maline and patchouli alcohol. Various other sesquiterpenes known are nardostachone, dihydrojatamansin, jatamansic acid (Bagchi, A, *et al*., 1990) jatamansinone, oroseolol, oroselone, seselin, nardostachyin, nardosinone, spirojatamol (Bagchi, *et al*.,1991) . jatamol A and B (Rucker, G,1993) calarenol (Sastry, S.D,1967) seychellene, seychelane, coumarin: xanthogalin. (Zinzius, J.1961) An alkaloid named actinidine has also been reported. Nardal has been found as an active component (Rucker, G. et al., 1978).

The present research focused on Medicinal properties of *Nardostachys jatamansi* **(Fig.1) (**family Valerianaceae), an original medicinal plant induces in organism a state of resistance against stress. It helps to promote physical and mental health augment resistance of the body against disease and has shown potent antioxidant activity. It has also shown marked tranquillizing activity, as well as hypotensive, hypolipidemic, antiischemic, hepatoprotective, neuroprotective, antioxidant activities (Rao VS, Rao A, Karanth KS, 2005; Lyle N *et al*., 2009).

Fig.1.*Nardostachys jatamansi* whole plant **Fig.2**.Dried Rhizomes of *Nardostachys jatamansi*

Nicotine is the primary psychoactive substance in tobacco that reinforces smoking behaviour (Henningfield and Goldberg, 1983). Nicotine from inhaled tobacco smoke is rapidly absorbed in the lungs and enters arterial circulation where it is then distributed to various body tissues, distributing in skeletal muscle, liver, spleen, lungs and brain, with a low affinity for adipose tissue (Hukkanen *et al.*, 2005). Nicotine also demonstrates a preferential partitioning (4 times) in brain over plasma (Ghosheh *et al.*, 2001). Nicotine is known to induced oxidative stress and depletes antioxidant defense mechanisms and produced reduction in glutathione peroxidase in skeletal muscle tissue, lung, liver and kidney of nicotine-treated

animals (Yildiz, 2004; Muthukumaran *et al.*, 2008). Nicotine also increases both free fatty acid release from the liver and the hepatic synthesis of very low-density lipoproteins; also maternal nicotine exposure induced oxidative stress and causes histopathological changes in the skeletal muscle tissue, lung and liver of lactating offspring (El-Sokkary *et al.,* 2007). Nicotine induces oxidative stress which may play an important role in the development of cardiovascular disease and lung cancer in smokers (El-Sokkary *et al.,* 2007). Nicotine may influence intracellular free $Mg2^{+}$ and Ca^{2+} concentrations via the influx of extracellular Ca^{2+} mainly across L-type voltage-gated Ca^{2+} channels, in a manner related to alpha-4-β-2 nicotine acetylcholine receptor (nAChR).Both are associated with process of synaptic plasticity in hippocampal neurons and these have been implicated in the pathology of central nervous system disorders. Including Alzheimers disease and schizophrenia. (Zhao, C; *et al*., 2009). The present investigation reveals that Nicotine toxicity effects on skeletal muscle ATPase effectively.

Skeletal muscles consist of hundreds to thousands, and sometimes millions, of long, multinucleated fibers organized together by an extracellular matrix. There are three general layers of extracellular matrix, or connective tissue, in muscles – the outermost layer is the epimysium, the intermediate layer is the perimysium and the inner most layer is the endomysium. Understanding the structure and function of each of these three layers requires a hierarchical approach. The structure and function of the epimysium and perimysium will be discussed in a whole body and tissue level biomechanical context, whereas the structure and function of the endomysium will be discussed in the context of cellular and molecular biomechanics. The ability of skeletal muscle and tendon to adapt to environmental changes, injury, illness and other physiological conditions is critical in determining the overall health. Skeletal muscle fibers have generally been categorized three types with the use of several histochemical techniques (pette and staron, 1990). Identification of muscle fibers as slow oxidative (SO), fast oxidative glycolytic (FOG), and fast glycolytic (FG) is based on the histochemical method using both mitochondrial enzyme and myosin adenosine triphosphatase (ATPase) activities to differentiate fibers (Barnard *et al.*,1971; Peter *et al.*,1972). A second method is based exclusively on the fibers' ATPase activity (Brooks and Kaiser, 1970) and it permits identification of one slow-twitch fiber (type I) and two fast-twitch fibers (types IIA and IIB). Analysis of single muscle fibers has demonstrated that specific myosin heavy chains I, IIa, and IIb correspond to the histochemically defined fiber types I, IIA, and IIB, respectively (Staron and Pette, 1986; Termin *et al.*, 1989).

In various muscle types, (Reactive oxygen species) ROS have been implicated in alteration of normal Ca^{2+} homeostasis via disruption of normal sarcoplasmic reticulum function. This may be proficient by inhibiting the Ca^{2+} ATPase pump and/or by activating the Ca^{2+} release channel. A number of studies have thorough the effects of one or more ROS on whole muscle tissue or on isolated sarcoplasmic reticulum consequent from smooth, cardiac, and/or skeletal muscles (Suzuki and Ford, 1991, 1992). Treatment of these tissues with ROS attempt to induce oxidative stress related to that experienced during ischemia and/or reperfusion. In smooth muscle, $O^{\cdot-}_2$ has been shown to inhibit both Ca^{2+}-ATPase activity and Ca^{2+} uptake into the Sarcoplasmic reticulum (SR) while stimulating inositol 1,4,5-trisphosphate-induced Ca release (Suzuki and Ford, 1991, 1992). In cardiac muscle, HOCl reduced contractile function and inhibited Sarcoplasmic reticulum (SR) Ca^{2+} uptake (Yanagishita, *et al.,* 1989). On a more microscopic level, H_2O_2 activated cardiac SR Ca^{2+} channel gating activity at mM concentrations. Superoxide also decreased Ca^{2+} uptake into cardiac Sarcoplasmic reticulum (SR) vesicles by increasing the Ca^{2+} permeability of the SR via opening the SR Ca^{2+} release channel (Okabe, 1991).

Reduced oxygen species may also interfere with skeletal muscle function. Significance of ischemic muscle, vitamin 'E' deficiency, and muscular dystrophy are important pathological conditions where ROS have been implicated in cytotoxic damage (Davison *et al.,* 1988). Despite this evidence, only a few studies have examined the direct effect of ROS on skeletal muscle function.

The aging process has been shown to result in an accelerated functional decline. The free radical theory of aging, however, has gained strong support because it is able to explain some of the processes that occur with aging and the degenerative diseases of aging. This theory proposes that an increase in oxygen radical production with age by mitochondria produce an increase in cellular damage (Harman, 1996, 1998). Aerobic organisms are well-protected against oxidative challenges by sophisticated antioxidant defense systems. However, it appears that during the aging process an imbalance between oxidants and antioxidants balance may occur, referred to as oxidative stress. Oxidative stress induced by oxidant species occurs under conditions when antioxidant defenses are depleted or when the rate constants of the radical reactions are greater than the antioxidant defense mechanisms (Buettner, 1993).

Aging reduces in skeletal muscle blood flow capacity. This decrease in flow capacity may be due, in part, to decreased endothelium-dependent vasodilation of the skeletal muscle resistance vasculature. Endothelium-mediated vasodilation to acetylcholine is reduced in arterioles from soleus but not gastrocnemius muscle of aged rats. (Muller-Delp, 2003). Some evidences implicate oxidative damage of cellular constituents in aging, as well as in the pathogenesis of the degenerative diseases of later years (Youngman *et al.,* 1992; Ames *et al.,* 1993; Viner *et al.,* 1996). Aging is related with a progressive decline

in muscle performance, characterized by decreased muscle strength and endurance capacity in both humans (Larsson *et al.*, 1979) and animals (Carmeli and Reznick, 1994). Although the reduction in muscle size could account for much of the reduction in muscle strength (Rodgers and Evans, 1993), the mechanism(s) underlying the reduced aerobic capacity is less clear. When animal models of aging have been used, a decrease of mitochondrial oxidative function as a cause of reduced aerobic capacity has been implicated by studies that show decreased oxidative enzyme activities in skeletal muscle homogenates (Stump *et al.*, 1977; Hansford, 1983) as well as in isolated mitochondria (Sugiyama *et al.*, 1993; Desai *et al.*, 1996). These discrepant findings with respect to age on skeletal muscle aerobic capacity could in part be explained by the different strains and species used in the studies as well as the different muscles sampled (Holloszy *et al.*,1991).

MATERIALS AND METHODS:

Animals:

Pathogen free, wistar strain male albino rats were used in the present study. The rats were housed in clean polypropylene cages under hygienic conditions with photoperiod of 12 hours light and 12 hours dark. The rats were fed with standard laboratory chow (Hindustan Lever Ltd, Mumbai) and water *ad libitum*.

Selection of Age group:

In the book, entitled "International care and treatment of rabbits, mice, rats, guinea pigs and Hamsters" published by W. B. Saunders Co., Philadelphia, USA., Schuchman (1989) given a detailed table regarding the age and life span of different strains of laboratory animals. As per this study the maximum life span of a rat is 3 years. Cao and Cutler, (1995) studied aging process from 6 months age through 12 and 24 months. However, between 12 months and 36 months the animal becomes older and with diminished physiological functions. On the basis of the physiology of the animal, in the present study "3 months age" group considered as "Young" and "18 months age" group was considered as "Old" for effective comparison of aging process .(Jang *et al.,2001*

Procurement of chemicals:

Nicotine and other fine chemical were obtained from Sigma chemical company, St. Louis, USA. All other chemicals and reagent used were of analytical grade.

Preparation of the *Nardostachys jatamansi* Extract:

100 grams of Jatamansi root powder [Indian Remedies, India] in 90 % ethanol [1L] at 50°C to 60°C in a Soxhlet extractor for 72 hours. The cooled liquid extract was concentrated by evaporating its liquid contents in rotary evaporator, with an approximate yield of 20%. The dried N.Jatamansi extract was suspended in distilled water, and used for the present study.

EXPERIMENTAL DESIGN:

Age matched rats were divided into 4 groups of six in each groups.

Group I – Normal Control: The rats were treated with normal saline [0.9%] orally via orogastic tube for a period of 2 months.

Group II – Nicotine treatment [Nt]: Rats were received the nicotine at a dose of 0.6 mg/kg body weight [0.5ml] by subcutaneous injection for a period of 2 months.

Group III – Nardostachys Jatamansi Extract treated [NJEt]: Rats were received N. jatamansi extract 50mg/kg body weight via orogastric tube for a period of 2 months.

Group IV – Nicotine + Nardostachys Jatamansi Extract treated [Nt+NJEt]:

These Rats were received the both nicotine [at a dose of 0.6 mg/kg body weight (0.5ml) by subcutaneous injection and N.jatamansi extract 50mg/kg body weight via or gastric tube for a period of 2 months. The animals were sacrificed after 24 hrs after the last treatment session by cervical dislocation and the skeletal muscle tissue were isolated and washed with ice-cold saline, immediately immersed in liquid nitrogen and stored at -80 ^{0}C for enzymatic assays. Before assay, the tissues were thawed, sliced and homogenized under ice-cold conditions. Selected parameters were estimated by employing standard methods.

BIOCHEMICAL ANALYSIS

ATPases : (ATP Phosphohydrolase) (E.C: 3.6.1.3) :

Mg^{2+} - ATPase :

ATPase activity was assayed by the method of Fritz and Hamrick (1966) as modified by Desaiah and Ho (1979). Tissue homogenates were prepared in ice cold 0.32 mM sucrose containing 1.0 mM EDTA and 10M imidazole (pH 7.5). The homogenates were centrifuged at 1000g for 15 minutes at 4^{0}C and the supernatant obtained was used as the enzyme source. The reaction mixture in a volume of 3 ml contained 3 mM ATP, 3 mM $MgCl_2$, 100 mM NaCl, 20 mM KCl, 135mM imidazole hydrochloric acid buffer (pH 7.5) and 10-30 μgm of protein as enzyme source. The reaction mixture was incubated at 37^{0}C for 30 minutes and the reaction was stopped by the addition of 0.1 ml of 50% TCA. Samples were then assayed for inorganic

phosphate using the method of Fiske and Subba Roa (1925). The colour was read at 660 nm in a spectrophotometer against the reagent blank. The Mg^{2+} – ATPase activity was measured in the presence of 1 mM ouabain, a specific inhibitor of Na^+ K^+ - ATPase. Ouabain sensitive Na^+K^+ - ATPase activity was obtained by the difference between total ATPase and Mg^{2+}- ATPase activity. The enzyme activity was expressed as μ moles of inorganic phosphate formed /mg protein/hour.

Ca^{2+} – ATPase :

Ca^{2+} – ATPase activity was determined by measuring the inorganic phosphate liberated during the hydrolysis of ATP. The reaction medium contained 135 mM imidazole-HCl buffer (pH 7.5), 5 mM $MgCl_2$, 0.05 mM $CaCl_2$, 4 mM ATP and 30-40 μgm of protein. The mixture was incubated at 37^0C for 30 minutes and the reaction was stopped by the addition of 0.1 ml of 50% TCA. The inorganic phosphate formed was estimated by the method of Fiske and Subba Row (1925). The colour was read at 660 nm against the blank in a spectrophotometer. Mg^{2+}-ATPase activity was measured in the presence of 0.5 mM EDTA and this value was subtracted from total ATPase activity to get Ca^{2+}-ATPase activity. Enzyme activity was expressed as μ moles of inorganic phosphate formed/mg protein/hour.

CREATINE PHOSPHOKINASE (CPK) (ATP creatine N-phosphotransferase):

E.C:2.7.3.2):

Creatine phosphokinase **(CPK)** activity was estimated by the method of Kuby *et.al.,* (1954), with slight modifications as given in the Sigma Technical Bulletin (1977) No.661. Ten percent homogenate of the muscle was prepared in ice cold distilled water and centrifuged at 1000g for 15 minutes. The supernatant fraction was used for enzyme assay. The reaction mixture in a final volume of 2.4 ml contained 60 μmoles of creatine, 100 μ moles of tris buffer (pH 9.0), 0.3 ml of the homogenate supernatant and remaining quantity of distilled water. The contents were thoroughly mixed and the tubes were placed in a water bath in 37^0C for a few minutes to warm up. The reaction was initiated by adding 5 μ moles of ATP and the contents were incubated for 30 minutes at 37^0C.

The reaction was under arrest by the addition of 1.6 ml of ice cold 20% (W/V) TCA and centrifuged. The inorganic phosphate formed was estimated by the method of Fiske and Subba Row (1925). To the supernatant 4.0 ml of distilled water, 1.0 ml of acid molybdate solution was added followed by the addition of 0.25 ml of amino-naptho sulphonic acid (ANSA). The contents were mixed well and allowed to stand for 30 minutes at room temperature to hydrolyse phosphocreatine and the colour developed was read at 660 nm in a spectrophotometer against the reagent blank. CPK activity was expressed as μmoles of inorganic phosphate liberated/mg protein/hour.

RESULTS AND DISCUSSION:

ATPases (Mg^{2+} and Ca^{2+} ATPases):

In the present investigation the ATPases (Mg^{2+} and Ca^{2+} ATPases) was significantly dropped ($P<0.01$) in both age groups (young and old) of nicotine treated rats (Changes in Mg^{2+} ATPases activity in young by -22.56%; old by -23.75% ; Changes in Ca^{2+} ATPases activity in young by -27.44%;old by -20.85 %) when compared to the control rats. In N.Jatamansi Extract treated (NJEt) rats of both age groups (young and old) significantly an elevation ($P<0.01$) was observed when compared to the control rats (Changes in Mg^{2+} ATPases activity in young by +29.76%; old by +25.36%). In the combination treatment (Nt+NJEt) non significantly an increase was observed when compared to the control rats of both age groups. (Table.1 & 2).

The ATPases have been classified based on the requirement of specific cations such as Mg^{2+}-ATPase, Na^+, K^+ - ATPase and Ca^{2+}-ATPase. All cells require energy to fulfill their diverse functions and to ensure the viability of the cells themselves and the entire organism. This energy is derived from the metabolism of nutrients, such as carbohydrates, proteins, and fats. When these nutrients are broken down, energy is released that is used to make ATP, which in turn can provide the energy to other reactions. Some forms of the nutrients can be stored in the cells so that they can be broken down to generate ATP whenever energy is needed for cellular reactions.

Mg^{2+}-ATPase has a specific role in the energy synthesis and is localized in mitochondria of all types of cells. It consists of 5 non-identical subunits (Christopher, 1979) which are the key components in oxidative phosphorylation (Boyer, 1976). Mg^{2+} – ATPase is an energy regulating enzyme intimately concerned with the oxidative phosphorylation. Na^+, K^+ - ATPase and Ca^{2+} – ATPase are considered to participate in the ATP hydrolysis in the physiological system. Na^+, K^+ - ATPase system has been termed as the enzymatic basis for active cation transport (Skou, 1957). This system has been demonstrated in brain (Deul and Mellwain, 1961; Aldridge, 1962; Samson and Quinn, 1967), Kidney (Whittam, 1962) and red cell membranes (Post *et al.*, 1960; Dunham and Glynn, 1961). Ca^{2+} – ATPase is one of the enzymes involved in hydrolysis of ATP and also plays a pivotal role in maintaining a constant intramolecular calcium concentration (Ohashi *et al.*, 1970). The hydrolysis of ATP by Ca^{2+} – ATPase involve a series of reactions in the formation of phosphorylated intermediates (Garraham and Rega, 1978). Because of the well demonstrated link to calcium ions transport (Schatzmann and Vinenchzi, 1969), the enzyme is also termed as the calcium ion pump ATPase.

In view of the important physiological functions of Ca^{2+} such as electrochemical coupling of muscle contraction, regulation of flow of metabolites across the mitochondrial membrane, maintenance of myofilament integration and tension development of the muscle fibre (Murray *et al.*,2000), the influence of Ca^{2+} on the activities of intracellular enzymes is especially interesting. In addition, the calcium is known to stimulate the activities of several enzymes (Nelson and Cox, 2001). Calcium ions are required for the muscle ATPase activity and also for phosphorylase (Murray *et al.*, 2000).

The decrease in the Mg^{2+} - ATPase during aging (Table.1) may be attributed to reduced oxidative metabolism and free energy formation. Aging process reduces ATP synthesis by affecting TCA cycle oxidation and respiratory chain. Decrease in Mg^{2+} activated (Rockstein and Brandt, 1962) and Ca^{2+} activated ATPase activities in gastrocnemius muscle of old rats (Syrovy and Gutmann, 1970) suggest more profound metabolic disturbances in the contractile machine. A decrease in oxidative enzyme activities and ATPase activities with a concomitant decrease in phosphorylase and glycogen content of the tissues in old age was reported by Talesara and Mohini (1978). They also reported a large decrease in the activities of all the enzymes in the aged rat kidney. Thus, the changes in the energy metabolism in muscles particularly with advancing age, may have an effect on its working capacity of the muscle ultimately. Decrease in the ATPase may be the result of age related decrease in number of contractile elements as revealed by low content of electrophoretically analysed myofibrillar proteins (Talesara and Rajni Arora, 1994).

In general the specific activities of Mg^{2+} - ATPase and Ca^{2+} -ATPase were elevated in the skeletal muscle tissue after N.Jatamansi Extract treated (NJEt) rats (Table.1 and 2). The increase in $Mg^{2+}$and Ca^{2+} -ATPases implied stimulation of a series of energy consuming reactions in intermediary metabolism and increased transport of $Mg^{2+}$and Ca^{2+} across cell membranes. Increase in Mg^{2+} and Ca^{2+} -ATPases enhances resistance to fatigue of low frequency stimulated muscle prior to elevations in aerobic oxidative capacity (Green *et al.*,1992). The increase in the specific activity of ATPases in general results in the hydrolysis of ATP which is utilized to overcome the energy demands during endurance N.Jatamansi Extract treatment.

Nicotine Toxicity can have profound negative effect on skeletal muscle tissues and their functions in maintaining the electrolyte balance. Nicotine has been shown to decrease ionic transfer through alterations in the monovalent cation pump and the antiport system. Nicotine particularly affects the concentrations of intracellular cations and ATPase activities.

CREATINE PHOSPHOKINASE (CPK):

Another important protein within the electrocyte is Creatine phosphokinase (CPK),' whose amino acid sequence is also highly homologous to the mammalian counterpart (**West, B. L *et al.*,1984;** Barrantes, F.J *et al*.,1985). Creatine phosphokinase (CPK) is used to transfer energy between phosphagens in the reversible reaction: In the present investigation the Creatine phosphokinase (CPK) activity was significantly decreased ($P<0.01$) in both age groups (young and old) of nicotine treated rats (Changes in CPK in young by -9.37%; old by -11.90%) when compared to the control rats. In N.Jatamansi Extract treated (NJEt) rats of both age groups (young and old) significantly an increase ($P<0.01$) was observed when compared to the control rats (changes in CPK in young by +17.34%; old by +18.72%). In the combination treatment (Nt+NJEt) non significantly an increase was observed when compared to the control rats of both age groups. (Table.3).

Creatine phosphokinase (CPK), is present in the cytosol and mitochondria of heart, skeletal muscle and brain of vertebrates. CPK activity is also found in tumours, adipose tissue, WBC and smooth muscles. CPK catalyses the reversible phosphorylation of ATP and creatine (Murray *et al.*, 2000). Like myosin ATPase, the key function of CPK is to replenish ATP, thereby supplying energy to muscle contraction.

Progressive decline in oxidative and glycolytic activities (Chen *et al.*, 1972) is the prominent features of aging which plays a dominant role in myopathies such as nutritional and hereditary muscular dystrophies and atrophies (Chengal Raju *et al.*, 1979). Hence, an attempt was made to study the activity pattern of CPK in selected skeletal muscle tissue which plays a essential role in the energetics of muscular contraction during the process of aging and continued existence of N.Jatamansi Extract treatment in male albino rats.

Creatine phosphokinase (CPK) is an enzyme present in many tissues, from which it may leak into the blood stream when tissue is damaged. This phenomenon is often used to detect tissue damage at an early stage to start treatment as soon as possible (heart) or to diagnose conditions which lead to muscle degeneration.

CPK was assayed in skeletal muscles tissue and were expressed as μ moles of pi/mg protein/hour. The CPK was greatly decreased in skeletal muscles tissue of young rats. The muscles of N.Jatamansi Extract treated rats of two age groups showed an augment in the CPK activity as compared to Normal controls. However, due to Nicotine treatment the CPK activity was decreased in the present study (Table.3).

The enzyme CPK catalyses reversible rephosphorylation of ADP by phosphocreatine to form ATP and creatine. A decrease in CPK activity was reported in several pathological conditions of muscles (Murray and Hoffmann, 1990). In the present investigation CPK activity was decreased in skeletal muscles tissue of nicotine treated rats with reference to aging which may be due to leakage of the enzyme from the muscle into serum as a consequence of muscle damage or loss of muscle mass or due to its decreased synthesis with advancement of age. Hence, the decline phosphorylation of creatine may also be one of the causes for the low CPK activity in muscle with reference to aging.

CONCLUSION:

The conclusion stated that the decrease in the ATPases (Mg^{2+}, Ca^{2+}) in nicotine treated rats with reference to aging may be attributed to reduce oxidative metabolism leading to reduced ATP synthesis due to reduced TCA cycle oxidation and respiratory chain efficiency. Creatine phosphokinase CPK which catalyses reversible rephosphorylation of ADP by phosphocreatine to form ATP and creatine showed decreased activity in the skeletal muscles tissue of rats which may be linked to the leakage of the enzyme from the muscles due to muscle damage or due to loss of muscle mass with advancement of age. Endurance N.Jatamansi Extract treated rat's improved utilsiation of creatine phosphate to resphosphorylate ADP to reimburse the depleted ATP levels. The increased CPK activity was to meet the increased energy demands

of working muscle. In the combination treatment (Nt+NJEt) of rats the CPK activity was initiate upregulations.

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Table – 1: Changes in **Mg^{2+}- ATPase** activity due to N.jatamansi Extract treated (NJEt), Nicotine treatment (Nt) and interaction of the two (NJEt+Nt) for a period of 2 months over the Control Skeletal muscles tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed as µmoles of inorganic phosphate liberated/mg protein/hour.

S.No	Name of the tissue	Young				Old			
		Control	Nt	NJEt	Nt+NJEt	Control	Nt	NJEt	Nt+NJEt
1	Skeletal muscle tissue	17.10 ±4.30	13.24** ±4.24 (-22.56)	22.19** ±5.12 (+29.76)	15.10@ ±4.24 (-11.71)	12.82 ±3.75	9.77** ±3.44 (-23.75)	16.07** ±5.43 (+25.36)	11.51@ ±3.66 (-10.24)

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
* Values are significant at $P < 0.05$
** Values are significant at $P < 0.01$
@ Values are non significant.

Table – 2: Changes in **Ca^{2+} – ATPase** activity due to N.jatamansi Extract treated (NJEt), Nicotine treatment (Nt) and interaction of the two (NJEt+Nt) for a period of 2 months over the Control Skeletal muscles tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed as μmoles of inorganic phosphate liberated/mg protein/hour.

S.No	Name of the tissue	Young				Old			
		Control	Nt	NJEt	Nt+NJEt	Control	Nt	NJEt	Nt+NJEt
1	Skeletal muscle tissue	25.01 ±5.70	18.15** ±6.26 (-27.44)	27.44** ±3.52 (+9.68)	23.17@ ±5.60 (-7.37)	22.22 ±3.95	17.59** ±4.23 (-20.85)	25.40** ±6.26 (+14.28)	20.01@ ±5.66 (-9.97)

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
* Values are significant at P < 0.05
** Values are significant at P < 0.01
@ Values are non significant.

Table – 3: Changes in **Creatine Phosphokinase** activity due to N.jatamansi Extract treated (NJEt), Nicotine treatment (Nt) and interaction of the two (NJEt+Nt) for a period of 2 months over the Control in skeletal muscles tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed as μmoles of inorganic phosphate liberated/mg protein/hour.

S.No	Name of the tissue	Young				Old			
		Control	Nt	NJEt	Nt+NJEt	Control	Nt	NJEt	Nt+NJEt
1	Skeletal muscle tissue	35.02 ±4.43	31.74** ±3.81 (-9.37)	41.1** ±4.01 (+17.34)	34.16@ ±3.66 (-2.46)	30.35 ±3.98	26.74** ±3.89 (-11.90)	36.04** ±4.28 (+18.72)	29.61@ ±3.40 (-2.45)

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
* Values are significant at P < 0.05
** Values are significant at P < 0.01
@ Values are non significant.