Antioxidant activity of *Nardostachys jatamansi* aligned with Nicotine induced stress on Skeletal muscle tissue of male Albino rat with reference to the Aging

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ABSTRACT

The roots and the rhizomes of *Nardostachys jatamansi*, are used to treat Muscle weakness, epilepsy, hysteria, mental and neurological disorders. Nicotine is highly addictive, It is one of the most commonly abused drugs, Several studies in humans and animal models provide evidence that nicotine does indeed result in muscle wasting. Age matched rats were be divided into 4 groups of six in each group and treated as follows: group i) Normal Control (NC) (Control rats received 0.9% saline) ; ii) Nicotine treated (Nt) (at a dose of 0.6 mg/ kg body weight by subcutaneous injection for a period of 2 months); iii) Nardostachys Jatamansiextract 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 by cervical dislocation. Isolated the skeletal muscle tissue and measured the activity levels of Superoxidedismutase (SOD), Catalase (CAT), Glutathione (GSH) and Glutathioneperoxidase (GPx). The decrease was observed in nicotine treated rats and increase was observed in NJEt rats over the control. . In the combination treatment (Nt+NJEt) up regulation was observed. In the present investigation, the antioxidant properties of *Nardostachys jatamansi*, are studied effectively.

Key words: Nardostachys jatamansi Extract, Nicotine, Skeletal Muscle tissue, SOD, CAT, GSH, GPx, and Male albino rat, Aging.

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INTRODUCTION:

The species *Nardostachys jatamansi* has very long history of use as medicine in Ayurveda, Homeopathy, ethno medicine and Indian System of Medicine (ISM) which is distributed in the Himalayas from Pakistan, India (Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Sikkim) to Nepal (Nayar MP, Sastry ARK, 1988). The rhizomes of the plant are used in Ayurvedic system of medicine as a bitter tonic, stimulant, antispasmodic, epilepsy and to treat hysteria **(**Polunin Oleg, Adam 1997**)**. The rhizomes and roots of the plant have medicinal value and, therefore, have been the focus of chemical studies (Hoerster H, Rucker G, 1977)**.** Chatterjee *et al*. undertook the chemical examination of the rhizomes in detail leading to the isolation of a new terpenoid ester, nardo-stachysin (Chatterjee A *et al.,* 2000). In the present investigation, the antioxidant properties and phenolic content of important indigenous drugs of India, *Nardostachys jatamansi,* used in neurological disorders like epilepsy, hysteria, syncope, convulsions, and mental weakness were studied. A number of studies suggest that oxidative stress plays an important role in the etiology of epilepsy and other neurological disorders.

The roots and rhizomes of *Nardostachys jatamansi* has been traditionally used in treatment of wide range of disorders, which include digestive system, circulatory system, nervous system, respiratory system, urinary system, reproductive system and skin diseases. It also exhibits cardio protective activity and used in the treatment of neural diseases. The essential oil obtained from the roots shows various pharmacological activities including antimicrobial, antifungal, hypotensive, anti-arrhythmic and anticonvulsant activity. Sesquiterpene is the major component of *N. jatamansi* plant, and also include jatamansone, nardostachone. In India, the rhizomes and roots are being marketed as an anticonvulsant Ayurvedic drug known as Ayush 56 and also used as an anti stress agent. The rhizome of *Jatamansi* is used as an aromatic adjunct in the preparation of medicinal oils, to promote hair growth and blackness. (Omprakash H.Nautiyal 2013). Neuronal hyperexcitability and excessive production of free radicals have been implicated in the pathogenesis of a considerable range of neurological disorders, including seizures and epilepsy. Oxidative injury may play a role in the initiation and progression of epilepsy. The large lipid content of myelin sheaths and the high rate of brain oxidative metabolism coupled with the low antioxidant Defenses make the brain highly vulnerable to free radical damage (Frantseva, M. V *et al.*, 2000). The roots of the herb is used in the preparation of an essential oil found to have fungi toxic activity, antimicrobial, antifungal, hy potensive, anti arrhythmic and anticonvulsant activity. The present article summarizes review on plant, its antioxidant properties.

Botanical classification:

Kingdom	Planate
Division	Mangnoliophyta
Class	Mangnoliopsida
Order	Dipsacales
Family	Valerianaceae
Genus	Nardostachys
Species	Jatamansi
Part used	Rhizomes, Rhizomes oil

Source: (India, 2014)

Botanical name: *Nardostachys jatamansi*

Nicotine is a stimulant and potent parasympathomimetic alkaloid that is naturally produced in the nightshade family of plants and used for the treatment of tobacco use disorders as a smoking cessation aid and nicotine dependence for the relief of withdrawal symptoms.("Nicotine" PubChem,2019). Nicotine is highly addictive It is one of the most commonly abused drugs. An average cigarette yields about 2 mg of absorbed nicotine; high amounts can be harmful. (Landoni JH. 2019) Nicotine induces both behavioral stimulation and anxiety in animals. Nicotine addiction involves drug-reinforced behavior, (Caponnetto P *et al*., 2012). Nicotine dependence involves tolerance, sensitization, physical dependence, and psychological dependence. Nicotine dependency causes distress.(Miyasato K March 2013) Nicotine withdrawal symptoms include depressed mood, stress, anxiety, irritability, difficulty concentrating, and sleep disturbances.(D'Souza MS, Markou A , July 2011). Today nicotine is less commonly used in agricultural insecticides, which was a main source of poisoning. More recent cases of poisoning typically appear to be in the form of Green Tobacco Sickness, accidental ingestion of tobacco or tobacco products, or ingestion of nicotine-containing plants. (Schep LJ, Slaughter RJ, 2009) People who harvest or cultivate tobacco may experience Green Tobacco Sickness (GTS), a type of nicotine poisoning caused by dermal exposure to wet tobacco leaves. This occurs most commonly in young, inexperienced tobacco harvesters who do not consume tobacco. (Schep LJ, Slaughter RJ, 2009). Nicotine has a higher affinity for nicotinic receptors in the brain than those in skeletal muscle, though at toxic doses it can induce contractions and respiratory paralysis. **(***Katzung BG 2006).*

Several studies in humans and animal models provide evidence that nicotine does indeed result in muscle wasting. For example, a 25% smaller fiber cross-sectional area was observed in the vastus lateralis muscle of smokers (Montes de Oca *et al*., 2008), even when matched for physical activity. The reduced

muscle fatigue resistance observed in smokers (Wu¨ st RC *et al*.,2008 a.) is hardly explicable by changes in fiber type composition or oxidative enzyme activity (Wu¨ st RC *et al*.,2008 b), another factor must cause the reduced muscle fatigue resistance in smokers. Such a situation could occur when the oxygen delivery to the mitochondria, or the ability of the mitochondria to use the oxygen, is impaired.

The skeletal muscles of vertebrates are made up of a mixture of tonic fibers (slow contraction), and twitch fibers (faster contraction) of which there are three different types. 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 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).

The different types of mammalian muscle fibers are not only unique in their myosin heavy-chain composition but also have relatively distinct physiological and biochemical properties (Burkey, 1981; Pette and Staron, 1990; Fuchtbauer, 1991). Mammalian muscles also contain varying proportions of the different types of fibers (Armstrong *et al.*, 1987). Therefore, it is not only important for investigators studying skeletal muscle to have an appreciation for the diverse characteristics of muscle fibers but also to know the fiber composition of specific muscles, muscle groups, or the entire body musculature. Skeletal muscle contributes significantly to multiple bodily functions. From a mechanical point of view, the main function of skeletal muscle is to convert chemical energy into mechanical energy to generate force and power, maintain posture, and produce movement that influences activity, allows for participation in social and occupational settings, maintains or enhances health, and contributes to functional independence. From a metabolic perspective, the roles of skeletal muscle include a contribution to basal energy metabolism, serving as storage for important substrates such as amino acids and carbohydrates, the production of heat for the maintenance of core temperature, and the consumption of the majority of oxygen and fuel used during physical activity and exercise. Of particular interest is the role of skeletal muscle as a reservoir of amino acids needed by other tissues such as skin, brain, and heart for the synthesis of organ-specific proteins (Wolfe RR , 2006). To illustrate the dynamic nature of muscle tissue we will also use the exercise/inactivity paradigm, aging, and some pathological conditions as important conditions that induce significant acute and chronic changes and adaptations in skeletal muscle. Some of these factors are discussed in more detail in other articles included in this issue of the journal.

Aging is associated 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 (Desai *et al.,* 1996). However, these findings are not supported in other studies, where no age-related changes in oxidative enzyme activities are found in skeletal muscle homogenates (Larkin *et al.,*1977; Skorjanc *et al.,*1998). Similarly, conflicting results were obtained in functional studies measuring the respiratory properties of isolated mitochondria, reporting either no changes (Farrar *et al.,*1981) or a decrease in respiratory rates with some but not with other substrates (Hansford, 1983). 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. Aging is associated with significant changes in the connective tissue compartment of skeletal muscle. An increase in both concentration of collagen and extent of nonreducible cross-linking occurs with aging in both skeletal muscle (Gosselin *et al.,*1994; Zimmerman *et al.,*1994) and heart (Thomas *et al.,*1992).

METERIALS AND METHODS

Animals:

Male pathogenic free wistar albino rats were obtained from the Department of Zoology, Animal House, S.V. University,Tirupati and Andhra Pradesh, India. The animals were housed six to each polypropylene cage and provided with food and water add libitum. The animals were maintained under standard conditions of temperature and humidity with an alternating 12hr light/dark. Animals were fed standard pellet diet [Agro Corporation Pvt. Ltd., Bangalore, India] and maintained in accordance with the guidelines of the National Institute of Nutrition and Indian Council of Medical Research, Hyderabad, India.

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 jatamansi ethanol 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 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 was 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} 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 INVESTIGATIONS:

Superoxide Dismutase: (SOD – EC: 1.15.1.6):

Superoxide dismutase activity was determined according to the method of Misra and Fridovich (1972) at room temperature. The skeletal muscle tissue was homogenized in ice cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v). The homogenates were centrifuged at 10,000 rpm for 10 min at 0^{0}C in cold centrifuge. The supernatant was separated and used for enzyme assay. 100 µl of tissue extract was added to 880 µl (0.05 M, pH 10.2, containing 0.1 mM EDTA) carbonate buffer; and 20 µl of 30 mM epinephrine (in 0.05% acetic acid) was added to the mixture and measured the optical density values at 480 nm for 4 min on a Hitachi U-2000 Spectrophotometer. Activity expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit.

CATALASE (CAT – EC: 1.11.1.6):

Catalase activity was measured by a slightly modified version of Aebi (1984) at room temperature. The skeletal muscle tissue was homogenized in ice cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v). The homogenates were centrifuged at 10,000 rpm for 10 min at 0^{0}C in cold centrifuge. The resulting supernatant was used as enzyme source. 10 µl of 100% EtOH was added to 100 µl of tissue extract and then placed in an ice bath for 30 min. After 30 min the tubes were kept

at room temperature followed by the addition of 10 µl of Triton X-100 RS. In a cuvette containing 200 µl of phosphate buffer and 50 µl of tissue extract was added 250 µl of 0.066 M H_2O_2 (in phosphate buffer) and decreases in optical density measured at 240 nm for 60 s in a UV spectrophotometer. The molar extinction coefficient of 43.6 M cm^{-1} was used to determine CAT activity. One unit of activity is equal to the moles of H_2O_2 degraded / mg protein / min.

GLUTATHIONE PEROXIDASE (GSH-Px – EC: 1.11.1.9):

Glutathione peroxidase (GSH-Px) was determined by a modified version of Flohe and Gunzler (1984). 5% (w/v) of skeletal muscle tissue homogenate was prepared in 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenates were centrifuged at 10,000 rpm for 10 min at 0^0C in cold centrifuge. The resulting supernatant was used as enzyme source. The reaction mixture consisted of 500 µl of phosphate buffer, 100 µl of 0.01 M GSH (reduced form), 100 µl of 1.5 mM NADPH and 100 µl of GR (0.24 units). The 100 µl of tissue extract was added to the reaction mixture and incubated at 37^0C for 10 min. Then 50 µl of 12 mM t-butyl hydroperoxide was added to 450 µl of tissue reaction mixture and measured at 340 nm for 180 s. The molar extinction coefficient of 6.22 X 10^3 M cm^{-1} was used to determine the activity. One unit of activity is equal to the mM of NADPH oxidized / mg protein / min. The enzyme activity was expressed in µ moles of NADPH oxidized / mg protein / min.

GLUTATHIONE (GSH) CONTENT:

Glutathione content was determined according to the method of Theodorus *et. al.* (1981). The skeletal muscle tissue were homogenized in 0.1 M ice cold phosphate buffer (pH 7.0) containing 0.001M EDTA and protein is precipitated with 1 ml of 5% sulfosalicylic acid (w/v) and the contents were centrifuged at 5000g for 15 min at 4^0C. The resulting supernatant was used as the enzyme source. The reaction mixture in a total volume of 2.5 ml contained 2.0 ml of 0.1M potassium phosphate buffer, 0.005 ml of NADPH (4 mg / ml of 0.5% $NaHCO_3$), 0.02 ml of DTNB (1.5 mg / ml), 0.02 ml of glutathione reductase (6 units / ml) and require amount of tissue source. The reaction was initiating by adding 0.41 ml of enzyme source and change in absorbance was recorded at 425 nm against the reagent blank. The glutathione content was expressed in nano moles / gram wet weight of the tissue.

STATISTICAL ANALYSIS

Statistical analysis has been carried out using INSTAT software. The data was analyzed for the significance; the results were presented with the P-values.

RESULTS AND DISCUSSION:

Superoxide Dismutase (SOD):

In the present study the Superoxide dismutase activity was significantly decreased (P<0.01) in both age groups (young and old) of nicotine treated rats (young by -32.35%; old by -31.07) when compared to the control rats. In N.Jatamansi Extract treated rats of both age groups (young and old) significantly an increase (P<0.01) was observed when compared to the control rats (young by +19.76%; old by +24.18 %). In the combination treatment (Nt+NJEt) non significantly an increase was observed when compared to the control rats of both age groups. (Table.1).

Superoxide Dismutase considered as front line of defense against the potentially cytotoxic free radical cause oxidative stress. The superoxide dismutase catalyzes the dismutation of two superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2) and oxygen. These enzymes obey first order reaction kinetics and the forward rate constants are almost diffusion limited. These results in steady state concentration of superoxide radicals in tissues that may be vary directly with the rate of superoxide generation and inversely with the tissue concentration of scavenging enzymes (Enghild *et al.*, 1999; Fattman *et al.*, 2003). In the present investigation a decline was absorbed in SOD activity in the skeletal muscle tissue of both age groups, due to nicotine treatment. The current results in the investigation are in consistence with the previous findings. SOD is an enzymatic antioxidant marker for oxidative stress, SOD level was highly significantly reduced in nicotine treated rats, when compared to control, however the old age rats also significantly reduced in compared to control. Similar study was found in cigarette smoking. (Kalra J *et al.*,1991). Chennaiah *et al.*, (2006) reported due to nicotine treatment SOD activity was decrease in the muscle tissue. The depletion of SOD activity was may be due to dispose of the free radical, produced by the nicotine toxicity. Khalindhar Basha *et al.*, (2013) reported in the kidney tissue the Superoxide dismutase activity was decreased due to nicotine toxicity. Helen *et al.*, (2000) reported the decreased SOD activity in brain tissue of rat due to nicotine toxicity. Nardostachys jatamansi Extract treatment (NJEt) produced a beneficial effect by decreasing the levels of oxidative stress in the mitochondria of skeletal muscle tissue. In the combination treatment (Nt+NJEt) upregulation of SOD activity was observed, reduce in oxidative stress and augmented activity of mitochondrial electron transfer enzymes, are logically related.

Catalase:

In the present study the Catalase activity was significantly decreased (P<0.01) in both age groups (young and old) of nicotine treated rats (young by -18.08%; old by -24.75) when compared to the control rats. In N.Jatamansi Extract treated rats of both age groups (young and old) significantly an increase (P<0.01) was observed when compared to the control rats (young by +26.24; old by +21.61%). In the

combination treatment (Nt+NJEt) non significantly increase was observed when compared to the control rats of both age groups. (Table.2)

Catalase is widely distributed in the body comportments, tissues and cell. In many cases the enzyme is located in subcellular organelles such *as,* peroxisomes and cytosol of liver (Atalay and Laaksonen, 2002; Lesiuk *et al.,* 2003). Mitochondria contain little amount of catalase. Catalase is a tetrameric peroxidative enzyme which converts the hydrogen peroxide to water and molecular oxygen and whose gene expression is regulated by H_2O_2. Catalase plays an important role in ROS metabolism and in adaptation to oxidant stress (Mates *et al.,* 1999; Vaziri *et al.,* 2003). Catalase catalysis the destruction of hydrogen peroxide into water and oxygen. Hydrogen peroxide is produced in the cells by a number of enzymatic reactions including those catalyzed by SOD, which converts superoxide anion radical to hydrogen peroxide and water (Fridovich 1995; Nordberg and Arner, 2001). CAT is an enzymatic marker to estimate oxidative level, CAT level was highly significantly reduced in Nicotine treated young age rats in compared to control, however the old age rats also significantly reduced in compared to control. Similar finding was found in cigarette smoker and tobacco users. (Zhang XY, 2007). The current results in the investigation are in consistence with the previous findings, Avati *et al.,* (2006) reported chronic administration of nicotine the CAT activity was decreased in the rat liver, lung and skeletal muscle tissue. The depletion of CAT activity was may be due to dispose of the free radical, produced by the nicotine toxicity. Das and Vasudevan, (2005b) reported a significant decrease in CAT activity with 2g /kg body weight ethanol treatment for a period of 4 weeks in hepatic tissue of Wistar strain male albino rats. This ethanol induced decrease in CAT activity may be due to enzyme protein oxidation as a result of accumulation of H_2O_2 and other cytotoxic radicals (Somani *et al.,* 1996). The decreased CAT activity with ethanol treatment indicates inefficient scavenging of hydrogen peroxide due to oxidative inactivation of enzyme. CAT is the main enzyme responsible for degradation of hydrogen peroxide in melanocytes (Maresca V, 2008). Nardostachys jatamansi Extract (NJEt) may capture the induced hydrogen peroxides before escaping it from the cell and breakdown them to water and oxygen. In this way NJEt can maintain the ample catalase activity in the skeletal muscle tissue under the induced oxidative stress condition. The upregulation in CAT activity was found with response of combination treatment of [Nt+NJEt] in the skeletal muscle tissue rats. The combination treatment augmented CAT activity in the skeletal muscle, suggesting that NJEt may help to develop a resistance in the skeletal muscle tissue to manage with nicotine induced oxidative injury and maintains the antioxidant system.

Glutathione perox1dase (gsh-px):

In the present study the glutathione peroxidase activity was significantly decreased (P<0.01) in both age groups (young and old) of nicotine treated rats (young by -14.43%; old by -22.74%) 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 (young by +19.67%; old by +14.64%). In the combination treatment (Nt+NJEt) non significantly an increase was observed when compared to the control rats of both age groups. (Table.3).

GSH-Px catalyzes the reduction of H_2O_2 or organic peroxides using reduced-glutathione as the electron donor to yield water or an alcohol along with oxidized glutathione (GSSH) (Halliwell and Gutteridge, 1999). GSH-Px can also terminate the chain reaction of lipid peroxidation by removing lipid hydro peroxides from the cell membrane (Sing and Pathak , 1999). In the present investigation we observed that decreased activities of GPx for these nicotine concentrations were observed, what may be explained by the redundant H_2O_2 level that cannot be eliminated. After treatment of cells with nicotine in lower concentrations, the activities of antioxidant enzymes, as well as the H_2O_2 content were similar to the controls. Increase of oxidative stress in melanocytes can be observed after exposition to nicotine in concentrations higher than 0.1 mM. The overproduction of ROS may cause damages in basic cellular components of cells resulting in dysfunctions or leading to cell death, Harmful effects of oxidative stress should be overcome by GPx.(Mari M,2010). Several studies have been reported by varies authors in different toxic conditions. Kazeem *et al.,* (2011) reported the GSH-Px activity was decreased in the hepatic tissue due to nicotine toxicity. Khalindhar Basha *et al.,* (2013) reported in the kidney tissue the Glutathioneperoxidase was reduced in nicotine treated rats. Ostrowska *et al.,* (2004) reported the decreased GSH-Px activity at a significant level in rat brain tissue for a period of 4 weeks ethanol intoxication. Decrease in GSH-Px activity may be due to either free radical dependant inactivation of enzyme or depletion of its co-substrate i.e., GSH and NADPH in the nicotine treatments. Similar studies, Santanukar Mahapatra *et al.,* (2008) reported smoking decreases the Glutathione peroxidase in the serum of mans. GPx works nonspecifically to scavenge and decompose excess hydro peroxides including H_2O_2, which may prevalent under oxidative stress (Somani *et al.,* 1996). In this study, decreased GPx activity seems to indicate the smoking induced oxidative stress. This investigation reveals that Nardostachys jatamansi Extract treatment (NJEt) enhanced skeletal muscle tissue glutathione peroxidase activity when compared to their respective controls. The elevation of glutathione peroxidase activity due to Nardostachys jatamansi

(NJEt) suggests an increased capacity to handle hydroperoxides in the skeletal muscl tissue. It appears that nardostachys jatamansi provide the required substrate for a high increase in the GSH-Px activity.

Glutathione (GSH):

In the present investigation the Glutathione content was significantly decreased (P<0.01) in both age groups (young and old) of nicotine treated rats (young by -28.10%; old by -20.14%) 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 (young by +17.96%; old by +32.26%). In the combination treatment (Nt+NJEt) non significantly an increase was observed when compared to the control rats of both age groups. (Table.4).

Glutathione is synthesized individually the constructive action of two enzymes, 1-GluCys synthetase uses glutamate and cysteine as a substrate forming the dipeptide. ϒ-GluCys which is combined with glycine in a reaction catalyzed by glutathione synthetase to generate glutathione. ATP is a co-substrate for both enzymes. The intracellular levels of GSH are regulated by a feedback inhibition of ϒ -GlyCys synthetase by the end product of GSH (Misra and Griffith, 1998). Therefore, cellular synthesis and consumption of glutathione balanced (Dringen, 2000). During detoxification of ROS, GSH is involved in two types of reactions. First, GSH reacts non-enzymatically with free radicals such as, the superoxide radical anion or hydroxyl radical (Singh *et al*., 1996) and second GSH is the electron donor for the reduction of peroxides in the GSH-Px reaction (Chance, 1979; Powers *et a1*., 2004).

In the present study we found that the administration of nicotine showing the decreased in GSH activity in the skeletal muscle tissue. Literature is not available related nicotine and skeletal muscle. However similar studies have been reported by several authors in different tissues. E.L Sokkary *et al*., reported chronic administration of nicotine the GSH activity was decreased in the rat kidney, liver and lung. Sener *et al*., reported chronic administration of nicotine the GSH activity was decreased in the rat tissues. Nicotine is oxidized primarily into its metabolite cotinine in the liver (Sastry BV, 1995), generates free radicals/ROS in tissues (Welscher G J, 1995), and induces oxidative tissue injury (Bhagwat SV *et al*., 1998). The decrease in GSH concentration in mitochondria would thus be highly responsible for ROS generation and the structural and functional damage in this organelle (Kannan M *et al*., 2004).

In the present study the GSH activity was increased in supplemented with Nardostachys jatamansi Extract (NJEt) in the skeletal muscle tissue of rats. Moreover, the percent elevation of GSH was more prominent in the skeletal muscle tissue in the combination treatment compared to control rats. Increased GSH content with NJEt may also due to the increase in the synthesis of precursors for GSH formation and increase the γ-Glutamyl- Cystineglycine enzyme, which is very essential for the GSH. The synthesis and

degradation of GSH is referred as the γ-Glutamyl cycle. This cycle small responsible for the enhanced GSH concentration in the skeletal muscle tissue with Nardostachys jatamansi Extract treatment. Glutathione peroxidase [GSH-Px] is a well-known first line defense of the cell against oxidative challenge, which inturn requires glutathione as a co-substrate. (Umadevi L, 1992).

CONCLUSION:

This investigation draws a conclusion stating that, *Nardostachys jatamansi* is an essential medicinal plant mentioned in Ayurveda and Unani system used for treatment of various diseases. *N. jatamansi* has many properties with animal studies which provide the researchers a platform to do research on those activities to scientifically legalize the finding and serve the humanity. Hence, the present study reveals that Nardostachys jatamansi Extract treatment (NJEt) may be beneficial, especially for the Nicotine subjects to improve Antioxidant enzyme status.

ACKNOWLEDGEMENTS:

The authors thankful to the Department of Zoology. Sri Venkateswara University. Tirupathi (A.P) for providing necessary facilities to carry out this work. The authors also highly grateful to the Dr.K.Chennaiah, Assistant Professor S.V.U.Tirupathi, for their constant Encouragement.

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Table–1: Changes in **Superoxide dismutase (SOD)** activity due to Nicotine treatment (Nt) , *Nardostachys jatamansi Extract* treatment (NJEt) and interaction of the both (Nt+NJEt) for a period of 2 months over the control in skeletal muscle tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed in units of Superoxide anion reduced/ mg proteins.

S.No	Name of the tissue	Young				Old			
		Control	Nt	NJEt	Nt+NJEt	Control	Nt	NJEt	Nt+NJEt
1	Skeletal muscle tissue	22.87 ±7.08	15.47** ±4.52 (-32.35)	27.39** ±6.01 (+19.76)	24.2@ ±6.59 (+5.81)	18.73 ±3.94	12.91** ±5.81 (-31.07)	23.26** ±5.25 (+24.18)	17.17@ ±5.61 (-8.32)

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 **Catalase (CAT)** activity due to Nicotine treatment (Nt) *Nardostachys jatamansi Extract* treatment (NJEt) and interaction of the both (Nt+NJEt) for a period of 2 months over the control in Skeletal muscle tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed in μ moles of H_2O_2 cleaved/mg protein/min.

S.No	Name of the tissue	Young				Old			
		Control	Nt	NJEt	Nt+NJEt	Control	Nt	NJEt	Nt+NJEt
1	Skeletal muscle tissue	31.02 ±8.31	25.41** ±7.60 (-18.08)	39.16** ±5.70 (+26.24)	29.10@ ±7.87 (-6.18)	28.32 ±5.70	21.31** ±7.29 (-24.75)	34.44** ±8.02 (+21.61)	26.38@ ±7.30 (-6.85)

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 **Glutathione peroxidase (Gp_x)** activity due to Nicotine treatment (Nt) , *Nardostachys jatamansi Extract* treatment (NJEt) and interaction of the both (Nt+NJEt) for a period of 2 months over the control in Skeletal muscle tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed in μ moles of thioether formed/ mg protein/min.

S.No	Name of the tissue	Young				Old			
		Control	Nt	NJEt	Nt+NJEt	Control	Nt	NJEt	Nt+NJEt
1	Skeletal muscle tissue	29.93 ±7.07	25.61** ±7.83 (-14.43)	35.82** ±8.78 (+19.67)	30.58@ ±8.92 (+2.17)	24.45 ±7.74	18.89** ±6.43 (-22.74)	28.03** ±7.11 (+14.64)	23.34@ ±6.22 (-4.53)

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–4: Changes in **Glutathione (GSH)** activity due to Nicotine treatment (Nt), *Nardostachys jatamansi Extract* treatment (NJEt) and interaction of the both (Nt+NJEt) for a period of 2 months over the control in Skeletal muscle tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed in *n* moles of glutathione/ gm/ wet wt of tissue.

S.No	Name of the tissue	Young				Old			
		Control	Nt	NJEt	Nt+NJEt	Control	Nt	NJEt	Nt+NJEt
1	Skeletal muscle tissue	65.85 ±7.87	47.34** ±8.67 (-28.10)	77.68** ±8.83 (+17.96)	56.22@ ±7.83 (-14.62)	48.84 ±7.89	39.00** ±7.51 (-20.14)	64.60** ±6.31 (+32.26)	49.39@ ±6.63 (+1.12)

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.