ISSN: 2377-6196
The American Journal of
SCIENCE AND MEDICAL RESEARCH, 2(3), 179-185

Research Article Open Access

Modulations in oxidative enzymes by *Nardostachys jatamansi* root extract against nicotine induced oxidative stress in the skeletal muscle tissue of male albino rat

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Abstract

Nardostachys jatamansi (Family:*Valerianaceae*) is a perennial herb found in Alpine Himalayas. *N. jatamans*i used for long period in various chronic diseases therapeutically. The species has very long history of use as medicine in Ayurveda. Family of *Solanaseace* consisting the nicotine in the number of plants. However, *Nicotiana tobacco* consisting more range of nicotine in the *Solanaseace family*. Nicotine it is harmful to the human beings. Pathogen free, wistar strain male albino rats were used in the present study. Wistar strain male albino rats were used in the present study. Rats were divided into 4 groups of six in each 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 Jatamansi extract treated (NJEt) (N.Jatamansi extract 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 as mentioned in Group II and Nardostachys Jatamansi as mentioned in Group III. The animals were sacrificed after 24 hrs after the last treatment by cervical dislocation and isolated the skeletal muscle tissue. The Succinate dehydrogenase (SDH) activity was decreased in nicotine treated,increased in NJEt and combination treatment rats. Malate dehydrogenase (MDH) activity was decreased in nicotine treated rats and increase was observed in the Nardostachys Jatamansi extract treated (NJEt) and combination treatment (Nt+NJEt) rats. The activity levels of LDH was increased in all treatment.In this study we have found out N.Jatamansi extract beneficial for the nicotine subject.

Key words: Nicotine, *N.Jatamansi* extract,SDH,LDH, MDH, Skeletal muscle and Male albino rat

INTRODUCTION

Nardostachys jatamansi (DC) is a small, perennial, rhizomatous, herb which grows in steep, moist, rocky, undisturbed grassy slopes of India, Nepal, China, Tibet and Bhutan from 2200 m to 5000 m above sea level (1). It is commonly known as jatamansi, Indian nard, balchar or spikenard. Its rhizomes are used in traditional medicines in different medicinal system (2). Jatamansi has been widely used for medicine and in perfumery for centuries in India. It is valuyed for many medicinal properties such as anti-lipid peroxidative hypolipidemic, antioxidant, hepatoprotective, sedative, tranquilizing, antihypertensive, anti-inflammatory ,antidepressant-like activity, anticonvulsant activity and hypotensive properties, anti-asthmatic and anti-estrogenic activity (3). Apart from this it is also used for the treatment of hair loss, growth and luster (4), and several nervous disorders such as epilepsy, neurosis, insomnia, excitation, alzheimers disease, learning and memory disorders (5,3). Their extracts also possess antispasmodic and stimulant properties which are useful in the treatment of fits and heart palpitations and it can also be used to regulate constipation, urination, menstruation and digestion (6).Due to several medicinal

How to Cite this Article:
K. Chennaiah, M. Jayachandrudu and J. Kamakshamma (2016). Modulations in oxidative enzymes by Nardostachys jatamansi root extract against nicotine induced oxidative stress in the skeletal muscle tissue of male albino rat. *The Ame J Sci & Med Res*, 2(3):179-185. doi:10.17812/ajsmr125.

Received: 2 June 2016; Accepted: 11 July 2016;
Published online 19 July 2016

properties, overexploitation has been carried out continuously and now the plant has entered in endangered status. Conservation Assessment and Management Plan (CAMP) workshops in India reported that observed population declines of 75-80% and classified jatamansi in different threatened categories. Hence such a multitude utility medicinal herb needs conservation; of biodiversity to maintain their population.

Jatamansi is known for several medicinal properties. It is most commonly used as a nervine sedative in the treatment of insomnia and also to treat chronic irritability and nervousness, with exhaustion and debility. Jatamansi primarily acts upon the nervous system, inducing a natural sleep, without any adverse effect upon awakening, and appears to lack the stimulating effects. Rhizome of jatamansi have also been used in traditional medicines as bitter tonic, stimulants, antipyretic, antispasmodic, antiseptic, anti-lipid peroxidative (7), anti-malarial (8), anti-rhythmic, sedative (9) antidepressant (10), diuretic, cardiac tonic tranquilizer, laxative, somachic, improve learning & memory (5) and also shows cytotoxic property (11). Extract of jatamansi is used in preparation of hair tonic, hair oils, promoting hair blackness, growth and luster (4). It is also used in oils and pasts that improve complexion and general health of the skin.

Nicotine is a naturally occurring alkaloid found primarily in the members of the *Solanaceae* family,which includes tobacco, potato, tomato, green pepper, and eggplant. Nicotine was first isolated and determined to be the major constituent of tobacco in 1828(12). In commercial tobaccos, the major alkaloid is nicotine, accounting for about 95 % of the total alkaloid content (13). Tobacco use is the leading cause of death in the world today. With 4.9 million tobacco-related deaths per year, no other consumer product is as dangerous or kills as many people as tobacco (14).

Nicotine, as most biologically active chemical in tobacco smoke, has been the subject of intense scientific scrutiny. Among the most well characterized chemicals found in tobacco and tobacco smoke, are polycyclic aromatic hydrocarbons (PAHs) and the highly addictive alkaloid, nicotine and its metabolites (15). To further complicate the picture, nicotine is converted, during the production of cigarette and chewing tobacco, into two highly mutagenic nitrosamine, *N*_-nitrosonor nicotine (NNN) and 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone (NNK) and is metabolized into cotinine. These chemicals derivatives also exhibit a wide spectrum of biological activity as compared to parent compound (15). Nicotine has been reported to induce oxidative stress both *in vivo* and *in vitro* (16). The mechanism of generation of free radicals by nicotine is not clear. But oxidative stress occurs when there are excess free radicals and/or low antioxidant defense, and result in chemical alteration of biomolecules causing structural and functional modification. Oxygen free radicals (OFR) production has been directly linked to oxidation of cellular macromolecules, which may induce a variety of cellular responses through generation of secondary metabolic reactive species (17). Medicinal plants and their active principles have received greater attention as anti-peroxidative agent (18).

Skeletal muscle is a form of striated muscle tissue which is under the voluntary control of the somatic nervous system. It is one of three major muscle types, the others being cardiac muscle and smooth muscle. Most skeletal muscles are attached to bones by bundles of collagen fibers known as tendons. Skeletal muscle is made up of individual muscle cells or myocytes, known as muscle fibers. They are formed from the fusion of developmental myoblasts (a type of embryonic progenitor cell that gives rise to a muscle cell) in a process known as myogenesis. Muscle fibres are cylindrical, and multinucleated. 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. This study was designed to investigate the effects of nardostachys Jatamansi extract on nicotine induced oxidative stress in the skeletal muscle tissue of male albino rat.

MATERIALS 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 [N.J] extract and mode of administration:

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 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 orogastric tube for a period of 2 months.

The animals were sacrificed after 24 hrs after the last treatment session by cervical dislocation and the kidney tissue were isolated at -4^0, 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 such as SDH,MDH and LDH by employing standard methods.

Biochemical Investigations

1. Succinate Dehydrogenase (SDH):

The specific activity of SDH was assayed by the method of Nachlas *et al.,*(19) as suggested by Prameelamma and Swami (20) with slight modifications. 10% homogenates of the skeletal muscle tissues were prepared in ice cold 0.25 M sucrose solution and centrifuged at 1000g for 15 minutes at 4^{0}C. The supernatant fraction was used for enzyme assay. The reaction mixture in a final volume of 2 ml contained 40 µ moles of sodium succinate, and 100 µ moles of phosphate buffer (pH 7.0) and 4 µ moles of INT. The reaction was initiated by adding 0.2 ml of homogenate containing 20 mg of tissue. The incubation was carried out for 15 minutes at 37^{0}C and the reaction was stopped by the addition of 5 ml of glacial acetic acid. The subsequent steps were followed same as described for LDH. The activity was expressed in µ moles of formazan formed / mg protein / hour.

2. Malate Dehydrogenase (MDH):

The specific activity of MDH was measured by the method of Nachlas *et al.,*(19) as suggested by Prameelamma and Swami (20) with slight modifications. 10% homogenates of the skeletal muscle tissues were prepared in ice cold 0.25 M sucrose solution and centrifuged at 1000g for 15 minutes at 4^{0}C. The supernatant fraction was used for enzyme assay. The total volume 2 ml of reaction mixture contained 100 µ moles of phosphate buffer (pH 7.0) 40 µ moles of sodium malate, 0.1 µ mole of NAD and 4 µ moles of INT. The reaction was initiated by the addition of 0.2 ml of homogenate containing 20 mg of tissue. The incubation was carried out at 37^{0}C for 30 minutes and the reaction was arrested by adding 5 ml of glacial acetic acid. The rest of the procedure was same as described earlier for LDH. The activity was expressed in µ moles of formazan formed / mg protein / hour.

3. Lactate Dehydrogenase (LDH):

Lactate Dehydrogenase activity was determined by the method described by Nachlas *et al.,*(19) as suggested by Prameelamma and Swami (20) with slight modifications. 10% homogenates of the skeletal muscle tissues were prepared in ice cold 0.25 M sucrose solution and centrifuged at 1000g for 15 minutes at 4^{0}C. The supernatant fraction was used for enzyme assay. The reaction mixture in a final volume of 2 ml contained 40 µ moles of sodium lactate, 100 µ moles of phosphate buffer (pH 7.4), 0.1 µ mole of NAD and 4 µ moles of INT. The reaction was initiated by the addition of 0.2 ml of homogenate containing 20 mg of tissue and incubated for 30 minutes at 37^{o}C and the reaction was stopped by the addition of 5 ml of glacial acetic acid. Zero time controls (ZTC) were maintained by addition of 5 ml of glacial acetic acid prior to the addition of the enzyme source to the incubation mixture. The formazan formed was extracted over night into 5 ml of toluene at 5^{0}C. The color developed was measured at 495 nm in a Spectrophotometer against the toluene blank. The enzyme activity was expressed in µ moles of formazan formed / mg protein / hour.

Statistical Analysis:

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

RESULTS & DISCUSSION

SDH is a key enzyme of Kreb's cycle which catalyses the reversible oxidation of succinate to fumarate. This enzyme is a flavoprotein with four iron atoms and four organic sulfides in addition to the flavin moiety. It is the only dehydrogenase in the TCA cycle which involves in the direct transfer of hydrogen atoms from the substrates to a flavoprotein without the participation of NAD. SDH is tightly bound to inner mitochondrial membrane. It serves as a link between electron transport system and oxidative phosphorylation.

The decrease(-23.45%)in SDH activity (Table-1) due to the nicotine stress condition indicates reduction in the conversion of succinate to fumarate resulting in decreased oxidative metabolism. During stress

Table–1. Changes in Succinate Dehydrogenase (SDH) activity due to Nicotine treatment (Nt), Nardostachys jatamansi extract treated (NJEt) and interaction of the both (Nt+NJEt) for a period of 2 months over the control in the Skeletal Muscle tissue of male albino rats. Values are expressed as μ moles of formazan formed/mg protein/hour.

S.No	Name of the tissue	Control	Nt	NJEt	Nt+ NJEt
1.	Skeletal Muscle	27.29 ±2.02	20.89* ±5.35 (-23.45%)	42.04* ±7.68 (+54.04%)	32.52** ±4.73 (+19.16%)

± SD of six individual observations.
Values in parentheses denote percent change over respective control.
*Values are significant at P < 0.001
** Values are significant at P < 0.01

Table–2. Changes in Malate dehydrogenase (MDH) activity due to Nicotine treatment (Nt), Nardostachys jatamansi extract treated (NJEt) and interaction of the both (Nt+NJEt) for a period of 2 months over the control in the Skeletal muscle of male albino rats. Values are expressed as μ moles of farmazan formed/mg protein/hour.

S.No	Name of the tissue	Control	Nt	NJEt	Nt+ NJEt
1.	Skeletal Muscle	53.45 ±5.40	44.12 * ±3.8 (-17.45%)	62.8* ±1.27 (+17.49%)	58.44@ ±1.33 (+9.33%)

± SD of six individual observations.
Values in parentheses denote percent change over respective control.
*Values are significant at P < 0.001
@Values are non significant at P < 0.05

Table–3. Changes in Lactate Dehydrogenase (LDH) activity due to Nicotine treatment (Nt), Nardostachys jatamansi extract treated (NJEt) and interaction of the both (Nt+NJEt) for a period of 2 months over the control in the Skeletal Muscle tissue of male albino rats. Values are expressed as μ moles of formazan formed/mg protein/hour.

S.No	Name of the tissue	Control	Nt	NJEt	Nt+ NJEt
1.	Skeletal Muscle	41.99 ±7.61	70.05 * ±1.57 (+66.82%)	45.46@ ±3.54 (+8.26%)	66.54* ±7.43 (+58.46%)

± SD of six individual observations.
Values in parentheses denote percent change over respective control.
*Values are significant at P < 0.001
@Values are non significant at P < 0.05

conditions diversion of phosphoenolpyruvate leads to increased formation of fumarate resulting in product inhibition of SDH (21). Chennaiah *et al*., (22) reported the decreased SDH activity was observed in all skeletal muscle fibers of rats treated with nicotine, indicating depressed oxidative metabolism in mitochondria. Since the activity of SDH is reduced, it is evident that this might affect the conversion of malate to oxaloacetate by MDH because of low succinate oxidation. A decrease in oxygen consumption in stress condition also leads to inhibition of mitochondrial oxido-reductases (23,24). The reduced availability of oxidized form of flavoproteins needed for succinate oxidation results in decreased activity of SDH (25).

In the present study an increase(+54.04%) was observed in the NJEt (Table-1) rats. The increase in specific activity of SDH in rats with response to NJEt suggests the increased mitochondrial oxidative potential and energy synthesis utilizing carbohydrates and fats as substrates function of mitochondria is energy production, isolated mitochondria generate reactive oxygen species during oxidative phosphorylation. Release of such

intermediates accounts for an estimated 1 to 5% of the oxygen consumed during respiration, depending on the substrate and respiration state. However, most studies used isolated mitochondria and the flux of oxidants was often estimated indirectly.In the combination treatment (Nt+NJEt) upregulation (+19.16%)was observed in the skeletal muscle tissue of rats. Thus differential response of SDH activity was observed in the skeletal muscle tissue of rats in the present study.

MDH catalyses the oxidation of L-malate to oxaloacetic acid using NAD as Cofactor and the reaction is reversible. Although the reaction is endergonic, it goes in the forward direction very rapidaly in the cell, because of the rapid removal of reaction products, oxaloacetate and NADH in subsequent steps. The activity of MDH depends on the rates of formation of oxaloacetate and phosphoenol pyruvate from malate. Any change in the mitochondrial structure inhibits the activity of MDH. The cytosolic and mitochondrial forms of malate dehydrogenases are key enzymes in the malate asparate shuttle (26).

The decrease (-17.45%) in specific activity of MDH (Table-2) in the skeletal muscle tissue due to nicotine treatment suggests decreased utilization of malate. The reduced levels of TCA cycle intermediates may also be due to the decrease in MDH activity during nicotine-treatment. Concisely, the decreased MDH activity could be attributed to 1) low availability of substrate, 2) lesser conversion of succinate-fumarate-malate, and 3) the changes in the structural integrity of mitochondria. An increase in proteolytic activity during nicotine intoxication may also be responsible for the decreased MDH activity. A similar study, Chennaiah *et al.,* (22) reported a decrease in specific activity of MDH was observed in the muscle fibers of rats treated with nicotine. The decreased MDH activity could be attributed to low availability of substrate, lesser conversion of succinate-fumarate-malate, and the changes in the structural integrity of mitochondria. Inhibition of MDH activity was observed in the present study suggests the prevalence of hypoxic condition in tissues and reduction in mitochondrial oxidative metabolism in tissues of rat administered with nicotine. The activity of MDH depends on the rats of formation of oxaloacetate and phosphoenolpyruvate from malate the reduced oxidation of malate evidenced by decreased MDH activity indicates the possible divertion of malate through Wood-workman reaction undergoing decarboxylation leading to the formation of phosphoenolpyruvate. Alterations in the activities of TCA cycle enzymes cause mitochondrial dysfunction and integrity ultimately leading to energy crisis during induced nicotine toxicity.

From the data it was observed, the activity levels of MDH were increased(+17.49%) in the skelatel muscle tissue in the NJEt rats. The increased MDH activity in response to N.Jatamansi suggests that higher utilization of malate. The elevation in MDH activity reflects the upturn of oxidative metabolism and the turnover of carbohydrates and energy output that is required during development (27). In the present study we report that the combination treatment (Nt+NJEt) exhibits a beneficial recovery(+9.33%) of MDH activity in the skeletal muscle tissue. This suggests that nardostachys jatamansi extract treated (NJEt) is very much useful for the nicotine subjects to upregulate the decreased oxidative metabolism (Table-2).

Lactate dehydrogenase is a key enzyme of anaerobic glycolysis and catalyses the reversible oxidation of lactate to pyruvate in the terminal step of glycolysis. The reaction catalysed by LDH interlinks anaerobic and aerobic oxidations of glucose. Any alteration in LDH activity indicates change in the production of pyruvate to lactate under anaerobic conditions favouring the reoxidation of NADH. This allows glycolysis to proceed in the absence of O_2 by generating sufficient NAD. In view of its role in glucose oxidation, NAD dependent LDH activity levels were assayed to assess the metabolic significance of this enzyme in compensatory mechanism operating in the tissues of rat during nicotine treatment, NJE treatment and combination treatment of Nt and NJEt.

In the present study rats witch received nicotine showed a elevation(+66.82%) of LDH activity in the skeletal muscle tissue. Chennaiah *et al.,* (22) reported a decrease in specific activity of LDH was observed in the muscle fibers of rats treated with nicotine. According to Yildiz *et al*., (28) LDH activity was increased due to nicotine induced oxidative stress. In our present investigations, the increased levels of LDH activity in nicotine treatments. This is due to the increased generation of ROS (Reactive oxygen species) by nicotine that leads to cell damage and also indicated the low capacity to combat against ROS.

The muscle tissue LDH activity was increased(+8.26%) in jatamansi treated rats when compared to control rats. This reports suggesting enhanced oxidative metabolism in NJEt rats to meet the increased energy demands of the animal. An increase in NAD dependent LDH activity in the skeletal muscle tissue of rat subjected to NJEt, indicate the possible shift in the metabolic profile from the anaerobiosis to aerobiosis i.e., the NAD-LDH activity helps in the efficient conversion of lactate to pyruvate and its subsequent utilization in TCA cycle oxidative reactions. The lactate taken up by the tissue may be oxidized to carbon dioxide and water or used for glycogenesis. In both cases pyruvate is the first product (29). Due to increased lactate levels in the skeletal muscle tissue, the LDH activity may also increase to convert the high amount of lactate to pyruvate during N. jatamansi extract treated rats (Table-3).

The lactate dehyclrogenase activity was elevated (+58.46%) in the combination treatrnent (Nt+NJEt) rats. According to Stuewe *et al.,* (30) increased glyceraldehyde dehydrogenase' may increase glycolysis and thereby elevated levels of lactic acid. The formation of pyruvate from lactate is catalyzed by lactate dehydrogenase, which is present in the sacroplasm at very high levels (29).This has lead to the proposal of an intracellular lactate shuttle, which involves transport of

lactate into mitochondrial matrix followed by oxidation to pyruvate, catalyzed by LDH (31).

Conclusion

This investigation draw a conclusion stating that this much of Nardostachys jatamansi extracts may be beneficial, especially for the nicotine subjects to improve the health status and life span.

Competing interests

The authors have declared that no competing interests exist.

References

1. Ghimire SK,Mckey D and Thomas YA. Conservation of Himalayan medicinal plants:Harvestging patterns and ecology of two threatend species, Nardostachys grandiflora DC. and Neopicrorhiza scrophulelriiflora (pennell) Hong.*Biological conservation* 2005; 124 (24):463-475.
2. ang YC. Tibetan Mataeria Medica. Qinghai Peoples Press, Qinghai,China(Ed).1996.
3. Rahman H, Shaik HA, Madhavi P, Eswaraiah MC. A review Pharmacognostics and pharmacological profiles of Nardastachys jatamansi DC. *Elixir pharmacy* 2011a; 39:5017-5020.
4. Bagchi A, Oshima Y, Hikino H. Neolignans and lignans of Nardostachys jatamansi root. *Planta Medica* 1991; 57:96-97.
5. Joshi H, Parle M. Nardostachys jatamasi impropves learning and memory in mice. Journal of *Medicinal food* 2006; 9(1):113-8.
6. Anon. Medicinal Plants of Nepal. Bulletin of the Department of Medicinal Plants No.3. His Majesty's Government of Nepal, Ministry of Forests and Soil Conservation, Department of Medicinal Plants, Thapathali, Kathmandu, Nepal 1993.
7. Tripathi YB, Ekta T,Anil U. Antilipid peroxidative property of Nardostachys Jatamansi. Indian Journal of *Experimental Biology1*996; 34:1150-1151.
8. Takaya Y, Kurumada K, Takeuji Y, Kim HS, Shibata Y, Ikemoto N, Wataya Y, Oshima Y. Novel antimalarial guaiane-type sesquiterpenoids from Naardostachys chinensis roots. *Tetrahedron Letters* 1998; 39:1361-1364.
9. Amin MG, Dixit YB, Pathak JD. Reaction time studies in relation to an indigeneous drug *Nardostachy jatamansi, Antiseptic* 1961; *58:565*
10. Dhingra D, Goyal PK. Evidences for the involvement of monoaminergic and GABAergic systems in antidepressant –like activity of Tinospora cordifolia in mice. *Indian Journal of Pharmaceutical Sciences* 2008a; 70(6) :761-767.
11. Itokawa H, Masuyama K, Morita H,Takeya K. Cytotoxic sesquiterpene from Nardostachys chinensis. *Chemical and Pharmaceutical Bulletin* 1993; 41(6):1183-1185.
12. Schevelbein H: Nicotine, resorption and fate. *Pharm Ther* 1982, 18:233-48.
13. Jacob P Yu L, Liang G, Shulgin AT, Benowitz NL: Gas hromatographic-mass spectrometric method for determination of anabasine, anatabine and ther tobacco alkaloids in urine of smokers and smokeless tobacco users. *J Chromatogr* 1993; 619:49-61
14. WHO. World Health Organization. Geneva. Available from:http:// www.who.int/ features/[2003] [cited 10 June 2005].
15. Campain JA: Nicotine: potentially a multifunctional carcinogen. *Topical* 2004; 79:1-3.
16. Pigeolot E, Corbisier P, Lambert D, Michiels C, Raes M, Zachary M O, Ramacle J: Glutathin peroxidase, superoxide dismutase and catalase inactivation by peroxides and oxygen derived radicals. Mech. Age. Dev 1990; 51: 283–297.
17. Chiarugi P. Reactive oxygen species as mediators of cell adhesion. Ital. J. *Biochem* 2003; 52:31-35.
18. Lee BM, Park KK. Beneficial and adverse effects of chemopreventive agents.*Mutat Res 20*03;523-524:265-78.
19. Nachlas MM, Morgulis SP, Seligman AM. A colorimetric method for the determination of succinate dehydrogenase activity. *J. Biol. Chem* 1960; **235:** 499-505.
20. Prameelamma Y, Swami KS. Glutamate dehydrogenase activity in the normal and denervated gastrocnemius muscle of frog Rana hexadactyla. *Curr.Sci* 1975; **44:** 739-740.
21. Moorthy KS. Modulation of carbohydrate and associated metabolism in the selected tissues of freshwater mussel, Lamellidens marginalise during induced methyl parathion stress, Ph.D. Thesis, S.V. University, Tirupati (AP), India 1983. .
22. Chennaiah K, Khalindar Basha K, Sivasankar R and Muneeswaraiah G. Changes in the Oxidative Metabolism Due to Nicotine Toxicity in the Skeletal Muscle Fibres of Male Albino Rat. *Journal of .Indian society of toxicology* 2011;00**7:**6-12.
23. Segel LD, Mason DT. Acute effects of acetaldehyde and ethanol on rat heart mitochondria, *Res. Commun. Chem. Pathol. Pharmacol* 1979; **25:** 461-474.
24. Moorthy KS, Kasi Reddy B, Swami KS, Chetty CS. Effect of the pesticide dichlorvos on succinate and malate dehydrogenase activities in fish, S. Mossambicus, *Environ. Ecol* 1985; **3:** 335-340.
25. Swami KS, Jagannatha Rao KS, Satyavelu Reddy K, Srinivasa Moorthy K, Linga Murthy K, Chetty CS, Indira K. The possible metabolic diversions adopted by the fresh water mussel to counter the toxic metabolic effects of selected pesticide, *Ind. J. Comp., Anim. Physiol* 1983; **1:** 95.

26. Chatterjea MN, Shinde R. Text book of Medical Biochemistry, Ist edition, Jaypee Brothers Medical Publishers Pvt. Ltd., New Delhi 1993; 258-280.
27. Murray RK, Grammer DK, Mayes PA, Rodwell VW. Harper's Biochemistry, Lang Medical Book, 25th edition, Appleton and Lange, *Prentice Hall Publ.* New Jersey, USA 2000 .
28. Yildiz D, LiuYS, Ercal N and Armstrong D. Comparison of pure nicotine- and smokeless tobacco extract-induced toxicities and oxidative stress. Arch. Environ. Contam. *Toxicol* 1999; **37**: 434 -439.
29. Rasmussen HN, Hall GV, Rasmussen UF. Lactate dehydrogenase is not a mitochondrial enzyme in human and mouse vastus lateralis muscle. *J. Physiol* 2002; **541(2):** 575-580.
30. Stuewe SR, Gwintz PA, Agarwal N,Mallet RT. Exercise training enhances glycolytic and oxidative enzymes in canine ventricular myocardium. *J. Mol. Cell. Cardiol* 2000; **32(6):** 903-13.
31. Brooks GA, Dubouchaud H, Brown M, Sicurello JP, Butz CE. Role of mitochondrial lactate dehydrogenase and lactate oxidation in the intraeellular lactate shuttle. Proc. *National Acad. Sci*. USA1999; **96:** 1129-1134.

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