



# Impact of *Nardostachys jatamansi* Extract and Nicotine on Creatine Phosphokinase Activity in Skeletal Muscle of Young and Old Male Albino Rats

## *Effects of N. jatamansi and Nicotine on Skeletal Muscle Creatine Phosphokinase*

Vanaja Kune and Kishore Salikineedy\*

Department of Zoology, Sri Venkateswara University, Tirupati – 517502. India,

**Abstract:** This study investigates the impact of *Nardostachys jatamansi* extract (NJEt) and nicotine (Nt) on creatine phosphokinase (CPK) activity in the skeletal muscle of young (3 months) and old (18 months) male albino rats. Over a period of two months, CPK activity was measured in both age groups. In young rats, skeletal muscle CPK activity was 35.02  $\mu\text{moles/mg protein/hour}$  in the control group, decreased to 31.74  $\mu\text{moles/mg protein/hour}$  with nicotine treatment, increased to 41.10  $\mu\text{moles/mg protein/hour}$  with NJEt treatment, and was 34.16  $\mu\text{moles/mg protein/hour}$  with the combined NJEt and nicotine treatment. In old rats, skeletal muscle CPK activity was 30.35  $\mu\text{moles/mg protein/hour}$  in the control group, decreased to 26.74  $\mu\text{moles/mg protein/hour}$  with nicotine treatment, increased to 36.04  $\mu\text{moles/mg protein/hour}$  with NJEt treatment, and was 29.61  $\mu\text{moles/mg protein/hour}$  with the combined NJEt and nicotine treatment. These results indicate significant alterations in CPK activity in response to NJEt and nicotine treatments, both individually and in combination. Nicotine treatment significantly decreased CPK activity in both young and old rats, while NJEt treatment led to a significant increase. The combined treatment of NJEt and nicotine mitigated the nicotine-induced reductions in CPK activity. Three-way ANOVA revealed significant interactions between tissue type, age, and treatment, indicating the complex interplay between these factors. The findings suggest that NJEt has a protective effect against nicotine-induced alterations in skeletal muscle, potentially due to its antioxidant properties.

**IndexTerms –** *Nardostachys jatamansi* extract; Nicotine; Creatine phosphokinase activity; Skeletal muscle; Male albino rats.

### I. INTRODUCTION

*Nardostachys jatamansi*, commonly known as spikenard, is a medicinal herb renowned for its therapeutic properties, including neuroprotective, antioxidant, and anti-inflammatory effects [1-3]. The bioactive compounds found in *N. jatamansi*, such as jatamansone and nardosinone, have been shown to mitigate oxidative stress and enhance cellular defense mechanisms [4,5]. These compounds contribute to the herb's ability to protect against cellular damage, promote neuroprotection, and support overall cellular health [6,7]. Conversely, nicotine, a major alkaloid present in tobacco, is well-documented for its harmful effects on health, particularly in relation to the musculoskeletal system [8,9]. Nicotine exposure has been associated with muscle atrophy, increased oxidative damage, and impaired muscle regeneration [10,11]. The detrimental effects of nicotine on muscle tissue are primarily due to its propensity to induce oxidative stress and disrupt normal cellular functions, leading to compromised muscle health and functionality [12]. Given these contrasting effects, it is critical to investigate the combined impact of *N. jatamansi* extract (NJEt) and nicotine on muscle biochemistry, specifically focusing on creatine phosphokinase (CPK) activity, an enzyme integral to energy metabolism in muscle tissues. Creatine phosphokinase (CPK) plays a pivotal role in the creatine phosphate energy shuttle, which is essential for maintaining cellular energy homeostasis, especially in tissues with high and fluctuating energy demands like skeletal muscles [13]. Variations in CPK activity are indicative of muscle damage, metabolic disturbances, and overall muscle health [14]. Previous studies have indicated that oxidative stress and muscle damage lead to altered CPK levels, which can serve as a biomarker for muscle integrity and function [15]. Therefore, understanding how NJEt, with its potent antioxidant properties, can influence CPK activity in the presence of a known muscle toxin like nicotine provides valuable insights into potential therapeutic interventions for muscle preservation and recovery.

Aging is accompanied by a natural decline in muscle mass and function, termed sarcopenia, which is further exacerbated by oxidative stress and a diminished capacity for muscle repair and regeneration [16]. This age-related muscle deterioration poses significant health challenges, increasing the risk of falls, frailty, and metabolic disorders [17]. By examining the effects of NJEt and nicotine on both young (3 months) and old (18 months) male albino rats, this study aims to elucidate age-dependent variations in

muscle response to these treatments. The differential impact on CPK activity in these age groups will provide critical insights into the potential of NJEt as a protective agent against nicotine-induced muscle damage and its efficacy in mitigating age-related muscle decline. This research not only advances our understanding of muscle biochemistry and aging but also explores the integration of traditional medicinal herbs in modern therapeutic strategies for enhancing muscle health and resilience. The findings from this study have the potential to inform the development of novel interventions aimed at improving muscle health and combating the adverse effects of both aging and nicotine exposure.

## II. MATERIALS AND METHODS

### 2.1 Animals

Male albino rats of Wistar strain, aged 3 months (young) and 18 months (old), were selected for the study. The animals were housed under standard laboratory conditions with a 12-hour light/dark cycle and were provided with a standard diet and water ad libitum. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC).

### 2.2 Experimental design

The rats were divided into eight groups, with six rats in each group:

- Group 1 (Young Control, SC): Young rats receiving standard diet and water.
- Group 2 (Young Nicotine, Nt): Young rats treated with nicotine.
- Group 3 (Young *N. jatamansi* extract, NJEt): Young rats treated with NJEt.
- Group 4 (Young NJEt + Nt): Young rats treated with both NJEt and nicotine.
- Group 5 (Old Control, SC): Old rats receiving standard diet and water.
- Group 6 (Old Nicotine, Nt): Old rats treated with nicotine.
- Group 7 (Old *N. jatamansi* extract, NJEt): Old rats treated with NJEt.
- Group 8 (Old NJEt + Nt): Old rats treated with both NJEt and nicotine.

### 2.3 Treatment regimen

- Nicotine Treatment (Nt): Nicotine was administered orally at a dose of 2 mg/kg body weight daily for two months.
- *N. jatamansi* Extract (NJEt): *N. jatamansi* extract was administered orally at a dose of 500 mg/kg body weight daily for two months.
- Combined Treatment (NJEt + Nt): Rats in the combined treatment groups received both nicotine and NJEt at the aforementioned doses for two months.

### 2.4 Sample collection

At the end of the treatment period, rats were sacrificed by cervical dislocation under anesthesia. Skeletal muscle tissues were rapidly excised, rinsed in ice-cold saline, blotted dry, and weighed. The tissues were then homogenized in 0.1 M phosphate buffer (pH 7.4) and centrifuged at 10,000 x g for 15 minutes at 4°C. The supernatant was collected and used for biochemical assays.

### 2.5 Biochemical analysis

**Creatine Phosphokinase (CPK) Activity:** CPK activity was measured in the supernatant using a standard spectrophotometric method. The assay involved the reaction of creatine phosphate with ADP to form creatine and ATP, catalyzed by CPK. The ATP formed was coupled to the production of NADH, which was measured at 340 nm. CPK activity was expressed as  $\mu$ moles of inorganic phosphate liberated per mg protein per hour.

### 2.6 Statistical analysis

Data were expressed as mean  $\pm$  standard deviation (SD) for six individual observations per group. Statistical significance was determined using three-way analysis of variance (ANOVA) to evaluate the effects of age, treatment, and their interactions on CPK activity. Differences were considered significant at  $P < 0.05$ .

## III. RESULTS AND DISCUSSION

### 3.1 Creatine Phosphokinase (CPK) activity

The study results, as summarized in Table 1 and Fig. 1, provide an overview of changes in creatine phosphokinase (CPK) activity in skeletal muscle (SM) tissue of both young (3 months) and old (18 months) male albino rats following a two-month treatment period with nicotine (Nt), *N. jatamansi* extract (NJEt), and their combination (NJEt + Nt). In young rats, the control group exhibited a CPK activity of  $35.02 \pm 0.68$   $\mu$ moles/mg protein/hour. Treatment with nicotine alone resulted in a significant reduction in CPK activity to  $31.74 \pm 0.93$   $\mu$ moles/mg protein/hour, reflecting a decrease of 9.37% ( $P < 0.001$ ). Conversely, administration of NJEt led to a significant increase in CPK activity to  $41.10 \pm 0.79$   $\mu$ moles/mg protein/hour, representing a 17.34% increase ( $P < 0.001$ ). The combination of NJEt and nicotine resulted in a CPK activity of  $34.16 \pm 1.09$   $\mu$ moles/mg protein/hour, which showed a non-significant decrease of 2.46%. For the older rats, the control group displayed a CPK activity of  $30.35 \pm 0.87$   $\mu$ moles/mg protein/hour. Nicotine treatment caused a notable decrease in CPK activity to  $26.74 \pm 0.69$   $\mu$ moles/mg protein/hour, an 11.90% reduction ( $P < 0.001$ ). Treatment with NJEt led to a significant increase in CPK activity to  $36.04 \pm 1.11$   $\mu$ moles/mg protein/hour, reflecting an 18.72% rise ( $P < 0.001$ ). The combination of NJEt and nicotine resulted in a CPK activity of  $29.61 \pm 0.81$   $\mu$ moles/mg protein/hour, showing a non-significant decrease of 2.45%. These findings highlight the varying effects of nicotine, NJEt, and their combination on CPK activity in different age groups of male albino rats.

**Table 1:** 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 muscle (SM) tissue of male albino rats of

young (3 months) and old (18 months) age groups. Values are expressed as  $\mu$ moles of inorganic phosphate liberated/mg protein/hour.

Name of the tissue	Young				Old			
	SC	Nt	NJEt	NJEt+Nt	SC	Nt	NJEt	NJEt+Nt
<b>Skeletal muscle tissue</b>	35.02 $\pm 0.68$	31.74** $\pm 0.93$ (-9.37)	41.10* $\pm 0.79$ (+17.34)	34.16@ $\pm 1.09$ (-2.46)	30.35 $\pm 0.87$	26.74* $\pm 0.69$ (-11.90)	36.04* $\pm 1.11$ (+18.72)	29.61@ $\pm 0.81$ (-2.45)

Three-way ANOVA for six observations per cell

Source of Variation	Sum of Squares	Degree of freedom	Mean sum of squares	F-values
Tissue (SM)	90.31	1	90.31	33.74**
Age (Young, Old)	130.66	1	130.66	30.96**
Treatment (SC, Nt, NJEt, NJEt+Nt)	505.87	3	168.99	29.83**
Age & Tissue	2.14	1	2.08	0.98@
Treatment & Tissue	13.88	3	5.44	1.04**
Treatment & Age	80.98	3	31.33	5.93**
Treatment & Age & Tissue	6.36	3	1.19	0.78@
Error	591.63	80	5.53	-
Total	1421.83	95	435.53	-

All the values are  $\pm$  SD of six individual observations.

Values in parentheses denote per cent change over respective sedentary control.

\* Values are significant at  $P < 0.001$

\*\* Values are significant at  $P < 0.05$

@ Values are non-significant.

### 3.2. Statistical analysis:

A three-way ANOVA was conducted to evaluate the effects of age, treatment, and their interactions on CPK activity. The results revealed significant effects for tissue type, age, and treatment ( $P < 0.05$ ). There were also significant interactions between treatment and tissue, and treatment and age ( $P < 0.05$ ), indicating a complex interplay between these factors.

The study investigated the impact of NJEt and nicotine (Nt) on creatine phosphokinase (CPK) activity in the skeletal muscle of young and old male albino rats (Table 1 and Fig. 1). The findings highlight significant alterations in CPK activity in response to NJEt and nicotine treatments, both individually and in combination. Nicotine treatment resulted in a significant reduction in CPK activity in both young and old rats. This decrease aligns with the well-documented detrimental effects of nicotine on muscle tissue, including increased oxidative damage, muscle atrophy, and impaired muscle regeneration [9]. The observed decline in CPK activity suggests that nicotine exposure disrupts muscle energy metabolism, contributing to muscle damage and compromised muscle function. In contrast, NJEt treatment significantly increased CPK activity in both age groups. *N. jatamansi* is known for its antioxidant properties, which likely contribute to the observed increase in CPK activity. The bioactive compounds in NJEt, such as jatamansone and nardosinone, are effective in mitigating oxidative stress and enhancing cellular defense mechanisms. This protective effect of NJEt suggests its potential as a therapeutic agent for preserving muscle integrity and function, particularly in the presence of muscle toxins like nicotine. The combined treatment of NJEt and nicotine resulted in CPK activity levels closer to the control values, indicating that NJEt can mitigate the nicotine-induced reductions in CPK activity. This finding underscores the protective role of NJEt against nicotine-induced muscle damage and suggests its potential in therapeutic strategies aimed at muscle preservation. The study also revealed age-dependent variations in muscle response to the treatments. The old rats exhibited lower baseline CPK activity compared to the young rats, reflecting the natural decline in muscle mass and function associated with aging (sarcopenia). The significant increase in CPK activity with NJEt treatment in old rats indicates its efficacy in countering age-related muscle decline and oxidative stress. The more pronounced decline in CPK activity with nicotine treatment in old rats further emphasizes the vulnerability of aged muscle to oxidative damage and metabolic disturbances. The findings of this study provide valuable insights into the biochemical effects of NJEt and nicotine on muscle tissue, highlighting the potential of NJEt as a protective agent against muscle toxins and age-related muscle decline. Further research is warranted to explore the molecular mechanisms underlying these effects and to evaluate the therapeutic potential of NJEt in clinical settings.

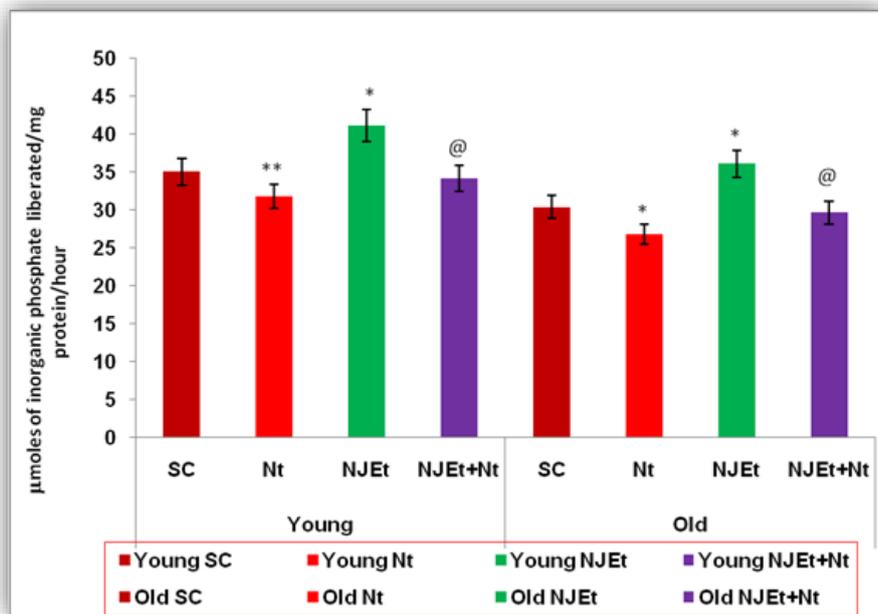


Fig. 1 Changes in Creatine Phosphokinase activity due to *N. jatamansi* Extract treated (NJEt), Nicotine treatment (Nt) and interaction of the two (NJEt+Nt) for a period of 2 months over the Control in skeletal muscle (SM) tissue of male albino rats of young (3 months) and old (18 months) age groups. Values are expressed as  $\mu$  moles of inorganic phosphate liberated/mg protein/hour.

#### IV. SUMMARY AND CONCLUSION

This study investigated the impact of NJEt and Nt on creatine phosphokinase (CPK) activity in the skeletal muscle of young and old male albino rats. Over a two-month period, the treatments revealed significant findings. Nicotine significantly reduced CPK activity in both age groups, underscoring its detrimental effects on muscle metabolism and integrity. Conversely, NJEt increased CPK activity, indicating its protective role against oxidative stress and muscle damage. The combined treatment of NJEt and nicotine showed that NJEt could mitigate nicotine's harmful effects, restoring CPK activity closer to control levels. The age-dependent variations highlighted that older rats had lower baseline CPK activity, reflecting age-related muscle decline, which NJEt effectively countered. In conclusion, the study demonstrates that nicotine negatively affects muscle CPK activity, contributing to muscle damage, while NJEt enhances CPK activity and provides a protective effect. The combined treatment results suggest NJEt's potential in countering nicotine-induced muscle damage. Moreover, NJEt's efficacy in ameliorating age-related muscle decline underscores its therapeutic potential for promoting muscle health and resilience, particularly in aging populations. These findings pave the way for further research into the molecular mechanisms and clinical applications of NJEt for muscle preservation and recovery.

#### V. ACKNOWLEDGMENT

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#### VI CONFLICT OF INTEREST

The authors do not have any conflict of interest.

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