

# STUDY OF SUBCHRONIC EFFECTS OF *Myristica fragrans* Houtt. on LDH and SDH levels of SWISS ALBINO MICE.

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**Abstract:** Plants and plant materials are consumed as condiments and spices, which not only enhance the flavors of food but also show preservative and medicinal properties. The spice Nutmeg- *Myristica fragrans* Houtt. has several medicinal properties. However it may prove to be toxic if consumed in larger quantities over a longer time period. Hence present paper aims at studying the sub-chronic effect of aqueous and methanolic extracts of Nutmeg on LDH and SDH levels of Swiss Albino Mice. When animals were treated for 28 days with aqueous and methanolic extracts of nutmeg, there is increase in serum LDH levels and decrease in serum SDH levels at  $p < 0.05$ . The increase in the Serum LDH activity and decrease in the SDH activity might be attributed to hyperglycemia. Increase in LDH levels might also be due to cellular damage or change in the membrane integrity caused due to the phytochemicals present in the nutmeg extracts.

**Keywords:** *Myristica fragrans* Houtt., Subchronic effect, LDH, SDH, Swiss Albino Mice

## I. INTRODUCTION:

Nature has gifted human population with wide range of plant resources. Most of these plant resources have medicinal values and hence play key role in human health care. A world health organization (WHO) survey indicated that about 70-80% of the world's population rely on non-conventional medicine, mainly of herbal source in their primary health care (1).

Some of these plants and plant materials are consumed as condiments and spices, which not only enhance the flavors of food but also show preservative and medicinal properties. Traditionally, there is a myth that herbs have been considered to be nontoxic and even harmless, mainly because of their natural origin. Though medicinal plant are nontoxic, it may prove to be toxic if consumed in large quantities or in an inappropriate portion. Nutmeg seeds - *Myristica fragrans* Houtt is used for various medicinal properties. The fruit, leaves and seed extract show various activities like hepatoprotective activity (2), antioxidant activity (3), memory enhancing activity (4), anticancer activity (5), antidiabetic activity, hypolipidaemic activity (6), antibacterial activity (7), and anti-inflammatory activity (8). Even though *Myristica fragrans* is an excellent source of medicinally active compounds and has diverse pharmacological effects, its consumption as a house hold remedy is not regulated. Since it is a potent aphrodisiac (9) it is consumed by males in larger doses and that too for a longer time span. The amount of nutmeg given to children to induce sleep or as an anti diarrhoeal agent (10) is never fixed or standardized.

Toxicant of any nature can cause ultrastructural change in mitochondria, endoplasmic reticulum and other cell organelles and change the enzyme levels depending upon the extent of cell injury or degradation. Long term consumption of nutmeg may cause toxic effect on the body and hence the present paper aims at studying the subchronic toxic effect of aqueous and methanolic extracts of nutmeg on the LDH and SDH levels of Swiss Albino Mice.

## Materials and Methods:

**Preparation of plant extracts:** Nutmeg seeds were procured from local market in Mumbai. Both Aqueous and Methanolic extracts of nutmeg were prepared by maceration technique (11, 12).

**Animals:** The animals used in the study, Swiss Albino mice *Mus musculus albinus* (20-30g), were purchased from the Haffkins Institute, and maintained in the Animal house of Ramnaraian Ruia College, Matunga, Mumbai. The animals were housed in groups of 5 in stainless steel cages (34×47×18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (Lipton), given water *ad libitum*. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Care and Use Committee of Ramnaraian Ruia College, Matunga, India.

**Experimental design:** The study was conducted on 40 animals as per the Guidelines No. 407 of OECD (13). The animals were divided into 8 groups. The animals were feed by oral gavage. The aqueous control groups were feed with 1mL of distilled water, whereas aqueous treated groups were feed with 1000mg/kg body weight of Aqueous extract of nutmeg. The methanol control groups were feed with 1 mL of olive oil whereas the methanol experimental groups were feed with 200 mg/kg body weight of Methanolic extract of nutmeg. The animals were grouped as follows:

Group I -Aqueous Control Female (ACF), Group II- Aqueous Treated Female (ATF)

Group III- Aqueous Control Male (ACM), Group IV-Aqueous Treated Male (ATM)

Group V- Methanol Control Female (MCF), Group VI- Methanol Treated Female (MTF)

Group VII- Methanol Control Male (MCF), Group VIII- Methanol Treated Male (MTM)

The animals were feed with the respective extracts for 28 days. On 29<sup>th</sup> day of the study, the animals were sacrificed by using high dose of ether anaesthesia. Blood samples were collected in heparinized tube by retro-orbital vein puncture method. Blood samples were centrifuged and serum was separated for the study of LDH (14) and SDH levels (15).

**Results:**

Results of sub-chronic effect of aqueous and methanolic extracts of nutmeg LDH and SDH levels of Swiss Albino Mice was expressed as mean ± SD, and n=06. Results were statistically analyzed using Student’s T test. P <0.05 were considered to be significant. The LDH and SDH levels of control and treated animals are represented in the observation table 1 and Figure 1 &2. There was significant difference in the LDH levels of animals treated with aqueous and methanolic extract (both males and females) in comparison to their respective control groups. No significant difference in LDH levels were recorded in ANM treated females (ATF) compared to ANM treated males (ATM) and MNM treated males (MTM) in comparison to MNM treated females (MTF). There was significant decrease in the SDH levels of animals treated with ANM and MNM (both males and females) in comparison to their respective control groups. No significant difference in SDH levels were recorded in ANM treated females (ATF) compared to ANM treated males (ATM) and MNM treated males (MTM) in comparison to MNM treated females (MTF).

**Table1. SUBCHRONIC EFFECT OF NUTMEG EXTRACTS ON LDH AND SDH LEVELS OF SWISS ALBINO MICE**

Groups	ACF	ATF	ACM	ATM	MCF	MTF	MCM	MTM
<b>LDH</b>	9.66 ±0.23	13.68 ±0.13	9.24 ±0.08	13.63 ±0.13	9.60 ±0.08	15.86 ±0.08	9.71 ±0.09	15.33 ±0.15
<b>SDH</b>	15.43 ±0.07	10.511 ±0.15	15.251 ±0.15	9.49 ±0.22	15.51 ±0.10	9.51 ±0.15	15.40 ±0.11	9.90 ±0.27

Values are Mean ± S.D. of 10 animals. Values are significant P ≤ 0.05

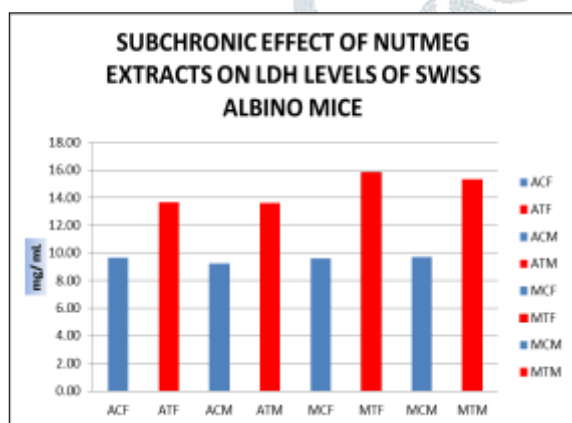


Figure: 1

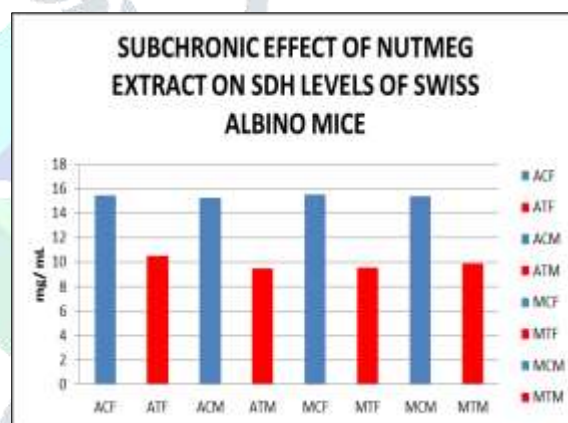


Figure:2

**Discussion:**

The succinate and lactate dehydrogenase are important enzymes of Krebs cycle whose qualitative changes are significant during certain pathological conditions. The percentage of alteration produced in the activity of these enzymes can be a physiological measure of the degree of inhibition of the glycolytic pathway and the normal metabolism of the animal. In present study the level of LDH is elevated and level of enzyme SDH has decreased in comparison to that of control in both groups treated with aqueous and methanolic extract of nutmeg at P< 0.05 level of significance.

In the study of DMBA (Dimethylbenz[a]anthracene induced skin carcinogenesis there was significant increase in LDH and decrease in SDH activity in liver and kidney of the mice (16). The rise in LDH activity in tissue suggested high turnover of pyruvate to lactate and vice versa to yield required energy to overcome DMBA induced oxidative stress and reactive oxygen species generation (17).

Increased LDH activity in diabetic rats has been reported by various researchers (18,19). Singh *et al.* reported that the elevated LDH levels in diabetic rats were associated with decreased insulin secretion (20). In diabetic animals, the extreme accumulation of pyruvate may lead to higher LDH activity. In the presence of LDH, excessive pyruvate is converted into lactate, leading to increased LDH activity, which could be attributed to the reduced insulin levels in diabetic individuals (21).

When female rats were treated with aqueous extracts of 500 mg/Kg of ginger, there was significant increase in serum LDH levels. The increase was apparently due to increased levels of cardiac LDH isozyme in serum (22)

In the present study the oxidative enzyme, SDH showed a reduction in its activity, which is an indication of suppression of oxidative metabolism in organisms exposed to toxicants. SDH is the oxidative enzyme involved in Krebs’ cycle. Since the cycle

represents a central oxidative pathway for carbohydrates, fats, and amino acids any disturbance in this enzyme activity the whole metabolism of the body is likely to be affected.

In support of present investigation, several authors reported a decrease in the activity of SDH after exposing to various toxicants. Decrease in the activity of SDH of gills, kidney, liver, muscle and brain tissues of fish *C. carpio* after exposing to pesticide phorate was reported (23). Studies have also indicated decrease in SDH activity of *Tilapia* in various tissues when animals were exposed to cycas seed extract (24)

It was reported that SDH activity decreased in STZ induced diabetic rats (25). The decreased SDH activity in diabetic condition is an indication of the depressed oxidative metabolism at the level of mitochondria.

Increase in LDH levels and decrease in the SDH levels in present study might be due to increase in blood glucose levels of the animal due to the oxidative stress generated by the nutmeg extracts. The increase in LDH levels can also be attributed to the cellular damage or changes in the membrane integrity caused by aqueous and methanolic extracts of nutmeg, which might have resulted in leakage of the enzyme. The decrease in SDH levels and increase in LDH levels might be one of the mechanisms favouring an aerobic metabolism in animals stressed due to exposure of the nutmeg extracts to meet energy demands.

#### Conclusion:

Nutmeg is considered to be a very good spice with respect to its flavoring capacity and medicinal properties. However it is suggested that long term consumption of nutmeg in larger doses may cause toxic effect on various physiological processes. The toxic effect of nutmeg can be attributed to the phytochemicals that are present in the extract.

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