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Nigella sativa Seeds Protective Ability in Pyrazinamide Induced Hyperuricemia in Mice

[1]Amtul Hafeez, [2]Abdul Mudabbir Rehan, [3]Zunera Hakim, [3]Attiya Munir, [4]Rabia Naseer Khan, [5]Aamna Khokhar

[1]*Department of Pharmacology, Islam Medical College, Sialkot*
[2]*Department of Pharmacology, D.G. Khan Medical College, Dera Ghazi Khan*
[3]*Department of Pharmacology, Rawalpindi Medical University, Rawalpindi*
[4]*Department of Pharmacology, Shahida Islam Medical College, Lodhran*
[5]*Department of Pharmacology, Islamabad Medical and Dental College, Islamabad*

ABSTRACT

Introduction: Hyperuricemia results in an increased level of blood uric acid, a prerequisite of gout. Commonly prescribed agents for the treatment of hyperuricemia include allopurinol, febuxostat, and probenecid. Multiple adverse effects like hypersensitivity, gastrointestinal upsets and hepatotoxicity limit their use.
Aims & Objectives: To evaluate the serum uric acid lowering effects of *Nigella sativa* seeds on pyrazinamide induced hyperuricemia in mice.
Place and duration of study: This study was carried out at research facility for animals of NIH, Islamabad and Pharmacology Department of Islamic International Medical College, Rawalpindi from April to June, 2017.
Material & Methods: Sixty-eight male mice (Swiss Albino) were separated into four groups. Group A mice were labelled as negative control and mice in this group were given chow & glucose water. Group B mice received 500mg/kg Pyrazinamide (PZA) added in glucose water once daily. Group C mice were given low dose *Nigella sativa* seeds powder in a dose of 500 mg/kg suspended in the glucose water accompanied by PZA in a dose of 500mg/kg. The mice in group D received high dose of *Nigella Sativa* seeds powder 1000 mg/kg suspended in glucose water along with 500mg/kg of PZA. All the doses of Pyrazinamide and *Nigella sativa* seeds suspension were given orally for six weeks. Blood sample was collected three times from each group. On day 0, sample from two mice from each group was taken for baseline uric acid levels and of five mice from all groups in mid of study to check uric acid levels. On 42nd day, the blood from remaining 10 mice in each group was taken to check the serum uric acid levels. Analysis of data was done using Graph Pad Prism Version 8, p value <0.05 was considered significant.
Results: Acute pyrazinamide administration caused a rise in uric acid levels in group B as compared to group A (from 5.94±1.94 to 28.03±15.52 mg/dl). The *Nigella sativa* seed powder extract suspended in glucose water in a dose of 500mg/kg and 1000mg/kg reduced the rise in uric acid levels in pyrazinamide treated group C and D (10.47±3.32 mg/dl & 7.53±1.78 mg/dl).
Conclusion: *Nigella sativa* possesses antihyperuricemic effect and showed a significant reduction in serum uric acid levels in a dose of 500mg and 1000 mg/kg.

Key words: *Nigella sativa,* serum uric acid (SUA), gout.

INTRODUCTION

The term hyperuricemia refers to clinical disorder in which there is an abnormal increase in the uric acid inside blood. In epidemiologic investigations, the cut of level of blood uric acid level in women is 6 mg/dl & about 7 mg/dl in men, Whereas an accepted reference level of 6.8 to 7.0 mg/dl depicts a theoretically soluble concentration of uric acid inside biological fluids.[1] The estrogen lowers uric acid level which protects premenopausal females from gout, that's why it makes hyperuricemia more common in males than in females.[2]

Hyperuricemia is a common problem due to several reasons including drugs. Many medications raise uric acid concentrations including diuretics, antitubercular drugs, calcinuerin inhibitors, anti-neoplastic and immunosuppressants. Hyperuricemia secondary to drugs can result from a decrease in the excretion or an increase in the production of uric acid.[3]

Uric acid is a heterocyclic organic compound with low solubility in water and plasma, whereas albumin is its main transporter.[4] It is an oxidative end derivative of purines. Xanthine oxidase catalyzes oxidation of hypoxanthine into xanthine and then

xanthine into the uric acid. Kidneys are the chief organs responsible for uric acid homeostasis. The amount of uric acid excretion is regulated by kidney via glomerular secretion and reabsorption.

Th net increase in either the production or decreased in the excretion of uric acid results in hyperuricemia. It is more prevalent in individuals who consume seafood, alcohol, and sweet beverages. Furthermore, drugs like pyrazinamide, ethambutol,[5] chlorothiazide, Ethacrynic acid, and salicylate can also elevate serum uric acid. Hyperuricemia is a common presentation in patients taking pyrazinamide with an incidence varying between 43.3 to 86.3%. Pyrazinamide induced hyperuricemia can result in acute gouty arthritis as well as moderate arthralgia. The hyperuricemic effect of pyrazinamide is attributed to its active metabolite pyrazinoic acid which decreases renal clearance of uric acid. It is reported to have trans-stimulatory effect on URAT1 causing reabsorption of uric acid from the luminal side into tubular cells.[6]

The deposition of urate crystals in the joint cavities results in gouty arthritis. The protein (animal source) is metabolized into oxalate & urate. The uric acid is responsible for the nucleation of calcium oxalate salts and that's why elevated levels of uric acid cause of formation of renal stones.[7]

Uric acid induces glomerular injury, tubule interstitial fibrosis and is responsible for causing metabolic syndrome.[8] Commonly prescribed drugs for the treatment of hyperuricemia are inhibitors of enzyme xanthine oxidase e.g., allopurinol & febuxostat whereas probenecid and benzbromarone reduce serum uric acid via their uricosuric mechanisms. Allopurinol causes diarrhea and severe cutaneous reaction[9] while febuxostat induces arthralgia and deranged liver enzymes.

The relative lack of drugs having antihyperuricemic effects, research work is in undergoing on classical medicinal plants. Flavonoids present in plants extracts possess xanthine oxidase inhibitory activity which is of great interest from biological research point. The xanthine oxidase enzyme inhibition is evident by plants such as *Cinnamomum cassia, Artemisia vulgaris, Onion* extracts*, H. lantanaefolia, Caesalpinia sappan, Lycopus europaeus* and *Allium Cepa*.[11]

Nigella sativa has been used for the treatment of different clinical disorders. The seeds and oil of *Nigella sativa* are frequently prescribed for treatment of many diseases such as acute and chronic cough, fever, extreme nasal congestion, chronic asthma, long standing diabetes mellitus, hypertension, severe eczema and inflammation, dizziness and gastrointestinal issues.[12] *Nigella sativa* has many pharmacological functions including antioxidant, anti-inflammatory, anticancer, antimicrobial, hypoglycemic, hypolipidemic, hepato-protective and spasmolytic.[13]

The therapeutic effects of *N. sativa* are considered to be due to the ingredient thymoquinone, a major active phytochemical present in oil.[14] Other constituents of *N. sativa* seed are natural carbohydrates and proteins along with the essential and fixed oil, alkaloids, sterols and saponins, crude fiber and organic acids, vitamins & minerals.[15]

The aim of this study is to observe the serum uric acid lowering effect of *Nigella sativa* seed powder suspension on pyrazinamide induced hyperuricemia in mice.

MATERIAL AND METHODS

A randomized controlled study was conducted for 3months (from April to June, 2017). The approval was given by Ethical Committee of faculty of Health and Medical Sciences, Riphah International University; Letter. No. Ripah/IRC/15/0116, Dated: April16, 2015. This study has been carried out at the animal house of NIH, Islamabad and Pharmacology Department of Islamic International Medical College, Rawalpindi. Blood sample evaluation was done at multi disciplinary research Lab of same college. Sixty-eight mice were divided into 4 groups containing seventeen in each group via non-probability technique.

Animals used: White male albino mice of 2 months age, having 25-35 grams weight were taken for study. Mice were kept under standard required conditions i.e., humidity 40-60%, temperature 20 ±20 C, & 12 hour light and dark cycle along with water and food *ad libitum.* Mice were acclimatized for 1 week before any intervention.

Chemicals:

Research grade salt of PZA was obtained from Pfizer Pharmaceuticals. *Nigella sativa* seeds were bought and certified by National Agriculture Research Centre (NARC), Islamabad. Electrical grinder was used to convert *Nigella sativa* seeds into fine powder. Glucose water was then added into powder to form Suspension. 1 gram *NS (Nigella sativa)* powder was added to 5ml of glucose water.

Preparation of pyrazinamide dosage form:

The 500mg PZA was mixed and suspended into 5ml of water containing glucose to get a homogenized solution. The PZA dose was calculated as per the body weight of mice.

Animal Groups:

Following 4 groups were formed. Chow and drugs were administered through oral gavage for 6weeks:

Group A: Animal in control group were given chow & glucose water

Group B: Mice of group B received 500mg/kg PZA added in glucose water per oral OD.[16]

Group C: Group C mice were given low dose *Nigella sativa* seeds powder in a dose of 500 mg/kg of *Nigella sativa* seeds powder dissolved in the glucose water along with 500mg/kg of Pyrazinamide (PZA)

Group D: This group was given a high dose of *Nigella sativa* seeds powder in a dose of 1000 mg/kg of *Nigella sativa* seeds powder dissolved in the glucose water along with 500mg/kg of Pyrazinamide (PZA).[16]

Blood samples collection:

Blood sample was collected three times from each group. On day 0, sample from two mice from each group was taken for baseline uric acid levels & of five mice from all groups on day 21of study for evaluating the changes. On 42nd day, remaining mice (10 in each group) were anaesthetized with chloroform and cardiac puncture was done to take a blood sample that was subjected later to the serum uric acid analysis Alinity Uric Acid Reagent Kit by Abbott was used for this purpose.

Statistical analysis:

Analysis of data was done using Graph Pad Prism Version 8. Quantitative data was given as Mean ± SD. The multiple comparisons among groups were done via the *Post hoc* Tukey test. The *p*-value of <0.05 was taken as research significant.

RESULTS

The mean uric acid levels ± standard deviation of all the groups on day 0, 21 and 42 are given in Table-1. The comparison (means values of all research groups) via ANOVA yielded a significant difference of means between groups with *p* value of <0.001 (Fig-1).

Serum Uric Acid levels						
Groups	Day 0		Day 21		Day 42	
	Mean	SD	Mean	SD	Mean	SD
Group A Negative Control	5.94	1.94	4.85	2.33	7.06	2.4
Group B Disease Control	28.03	15.52	7.4	0.84	13.16	6.03
Group C Experimental 500 mg/kg	10.4	3.32	4.2	1.76	8.98	4.39
Group D Experimental 1000mg/kg	7.5	1.78	2.55	0.77	5.04	2.39

Table-1: Serum uric acid values on day 0 (n=2), day 21 (n=5) and day 42 (n=10)

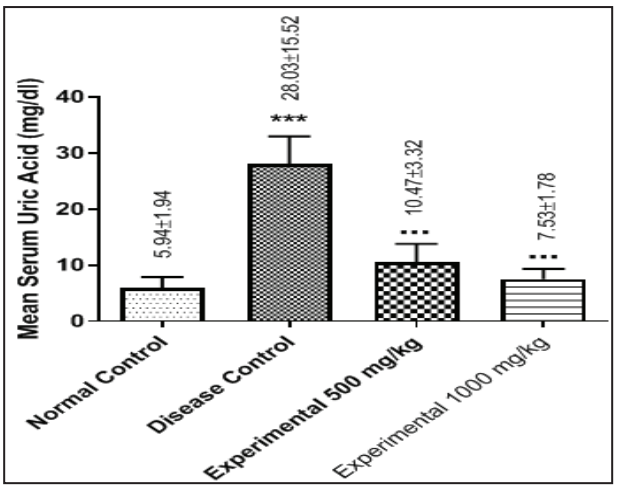

Fig-1: Effect of Pyrazinamide and Pyrazinamide plus 2 different doses of Nigella sativa seeds powder on uric acid levels (Mean ±SD) in mice (n=10) on day 42.
***=*p*-value <0.001 (vs Group A, the Normal Control)
… = *p*-value <0.001 (vs Group B, the Disease Control)

The serum uric acid level difference in between groups was evaluated via the *post hoc* Tukey's test. The disease control group had higher levels of serum uric acid as compared to the normal control, whereas the experimental groups had markedly significant lower levels of serum uric acid as compared to the disease control group (Table-1).

Both the experimental groups had high serum uric acid levels as compared to normal control which was statistically non-significant. The difference among 2 doses of Nigella sativa seed powder was statistically non-significant.

DISCUSSION

Hyperuricemia is present in up to 18% of the general population and is predominant in males than in females.[17] The uric acid is final product of the purine nucleotide catabolism[18] and hyperuricemia occurs from either increase in production or decrease in excretion of uric acid. Allopurinol remains to be the dominant xanthine oxidase inhibitor available to treat hyperuricemia, but has limited utilization due to related adverse effects.[19] Other uricosuric agents like probenecid & sulfinpyrazone are nephrotoxic. The benzbromarone use is associated with severe fulminant liver toxicity.[20] Thus, the quest for uric acid lowering agent is highly necessary.

Phenolic compounds in fruit of *Nigella sativa* are present in dried fruit and it is very good source of flavonoids which possesses excellent antioxidant properties.[21]

Pyrazinamide treatment caused a twofold increase in the levels of serum/blood uric acid. On the 42nd day, serum concentration of uric acid significantly

declined (p-value < 0.001) in both experimental groups as compared to the positive control. Similar studies representing the serum uric acid lowering effect of *Nigella sativa* are not available, that's why research studies performed on other medicinal herbs were considered as reference to compare the results of current study e.g., the result of current research are comparable to the work accomplished by Haideri et al[22] and the researchers determined that the onion juice reduced uric acid concentration significantly with a $p$value < 0.001 in hyperuricemic rats both in time & dose-dependent manner. Similarly, the current results are in consensus with the effects of the crude flavonoid compound of *Zingiber officinale*[23] and the essential oils of the leaves of the *Cinnamomum Osmopholieum*[24] which significantly suppressed the high uric acid concentration in the hyperuricemic rats in the dose dependent manner only ($p$value < 0.001).

The proposed mechanism of hyperuricemia caused by pyrazinamide is due to its strong urateretention ability.[25] Pyrazinamide also increases serum uric acid by the trans stimulatory effect on URAT1causing the reabsorption of uric acid from luminal side to tubular cells.[26] Pyrazinamide also inhibits the OAT2 (a protein present in basolateral membrane of PCT (proximal tubule cell) and is responsible for the secretory transport of urate).[27] OAT2 is a possible target of antiuricosuric effects of the pyrazinamide as well as the URAT1.

Nigella sativa contain some important compounds that help to improve renal health as phenolic compounds, flavonoids, minerals, and vitamins diminish uric acid levels and keep a safe kidney from damage, the mechanism underlying this effect is probably their molecular structure. These antioxidants act as direct superoxide scavengers & xanthine oxidase inhibitors, resulting in the suppression of Reactive Oxygen Species (ROS) and uric acid formation.[28] Thus, *Nigella sativa* reduces the uric acid formation and maintains normal levels of uric acid whenever PZA interferes the uric acid levels.

The greatest strength of this study is that it is natural and cost-effective way to keep uric acid levels in normal range in patients taking PZA. The limiting factors of this study is the lack of parallel comparison with the similar studies. Additionally, no control group of known antihyperuricemic drug was taken into account to compare the significance of antihyperuricemic effect of Nigella sativa

In this study, two different doses of *Nigella sativa* were selected to demonstrate its hypouricemic effect. The study results revealed that *Nigella sativa* seed powder significantly lowered serum uric acid level in a dose of 500mg/kg and 1000mg/ kg.

CONCLUSION

Nigella sativa seed powder has the potential to lower serum uric acid levels and it produced a significant anti hyperuricemic effect in a dose of 500mg/kg and 1000mg/kg.

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The Authors:

Dr. Amtul Hafeez
Assistant Professor,
Department of Pharmacology,
Islam Medical College, Sialkot.

Dr. Abdul Mudabbir Rehan
Assistant Professor,
Department of Pharmacology,
D. G. Khan Medical College, Dera Ghazi Khan.

Dr. Zunera Hakim
Assistant Professor,
Department of Pharmacology,
Rawalpindi Medical University, Rawalpindi.

Dr. Attiya Munir
Assistant Professor,
Department of Pharmacology,
Rawalpindi Medical University, Rawalpindi.

Rabia Naseer Khan
Assistant Professor,
Department of Pathology,
Shahida Islam Medical College, Lodhran.

Aamna Khokhar
Assistant Professor,
Department of Pharmacology,
Islamabad Medical and Dental College, Islamabad.

Corresponding Author:

Dr. Abdul Mudabbir Rehan
Assistant Professor,
Department of Pharmacology,
D. G. Khan Medical College, Dera Ghazi Khan.
E-mail: abdulmudabbir@yahoo.com