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EVALUATION OF THE BENEFICIAL EFFECTS OF ASPIRIN AND ATORVASTATIN SEPARATELY AND COMBINED ON HYPERURICEMIC RAT MODEL

Dr. Usman Aslam[1], Dr. Ayela Eman Zia[2*], Dr. Arooj Fatimah[3], Dr. Waqar Ahmed Siddiqui[4], Dr. Sidra Zahid[5], Dr. Gulpash Saghir[6]

[1,2*]Assistant Professor, Department of Pharmacology, CMH Lahore Medical College, Lahore, Pakistan
[3,5]Senior Demonstrator, Department of Pharmacology, CMH Lahore Medical College, Lahore and Pakistan
[4]Associate Professor, Department of Pharmacology, CMH Lahore Medical College, Lahore, Pakistan
[6]Associate Professor of Pharmacology, Fatima Jinnah Medical University, Lahore, Pakistan

***Corresponding author:** Dr Ayela Eman Zia
*Email: ayelaemanz@gmail.com

ABSTRACT

Introduction: Low doses of aspirin and statins are commonly prescribed together for patients with coronary artery disease. Aspirin at low doses is known to cause hyperuricemia in these patients while there is some evidence that atorvastatin may have a hypouricemic action but underlying mechanism remain speculative. In this study we will investigate the effects of atorvastatin and aspirin alone and in combination on serum uric acid of hyperuricemic rats. **Aims & Objectives:** To estimate the effect of atorvastatin and low dose of aspirin alone and in combination on serum uric acid level and urinary uric acid excretion in hyperuricemic rats. **Place and duration of study:** Post Graduate Medical Institute, Lahore from November 2019 to April 2020. **Material & Methods:** Thirty rats were divided into five groups of six rats each. Group I and II were given distilled water, III, IV and V were given aspirin alone, atorvastatin alone and combination of aspirin and atorvastatin, respectively, as a single morning dose for 7 days. Group I was given normal saline and group II, III, IV and V was given potassium oxonate on day 1, 3 and 7. One ml blood and twenty-four-hour urine sample was collected on week 0 and 4 for estimation of uric acid and creatinine concentrations. Fractional excretion of uric acid was calculated **Results:** The serum uric acid levels rose significantly in disease control and all other experimental group between day 0 and 7. Between day 0 and 7, there was no significant change in urinary uric acid level of normal control group, while the values became significantly high in all other groups between day 0 and 7. There was significant rise in urine creatinine of aspirin and atorvastatin combined group between day 0 and 7. The percentage decrease as compared to day 0 was highest in atorvastatin group followed by combined aspirin and atorvastatin group and aspirin alone treated group.

Conclusion: The results could not affirm the hypothesis of nullifying the hyperuricemia effect of low dose aspirin with the addition of atorvastatin in the hyperuricemic rats.

Keywords: Hyperuricemia, Aspirin, Atorvastatin

INTRODUCTION

Hyperuricemia, characterized by elevated levels of serum uric acid (SUA), either due to overproduction or underexcretion of uric acid, is associated with spectrum of commodities such as gout, renal disorders, cardiovascular diseases, diabetes and metabolic syndrome etc. The prevalence of hyperuricemia is on the rise worldwide and it significantly lowers the quality of life for people who are affected (Du et al.,2024). In clinical practice, drug-induced gout and hyperuricemia present an emergent and increasingly prevalent issue. These drugs include anti-tubercular, diuretics, testosterone, xylitol, cytotoxic chemotherapy, immunosuppressive agents, nicotinic acid and aspirin (low doses) (Ben Selam et al., 2017). Aspirin, an irreversible inhibitor of COX in platelets, has a two-way effect on uric acid excretion (Aslam et al., 2019). Numerous studies found that serum uric acid (SUA)levels were increased under low-dose aspirin due to inhibited uric acid excretion (Zhang et al., 2020) At high doses aspirin has uricosuric effect (Kaufmann et al., 2024).

Prior research has demonstrated some evidence linking elevated SUA levels to increased risks of cardiovascular conditions, including peripheral vascular disease, ischemic heart disease, stroke, diabetes, hypertension, heart failure, obesity, and renal failure (Shahin et al., 2021). Hyperuricemia is associated with the risk of coronary heart disease mortality. Elevated SUA can cause endothelial dysfunction, oxidative stress, and inflammatory responses. In addition, hyperuricemia may also induce the production of oxygen free radicals and platelet adhesiveness (Zuo et al., 2016). Low-dose aspirin is the cornerstone of secondary prophylaxis of arteriosclerotic cardiovascular disease. Nevertheless, other investigations found that low-dose aspirin may increase SUA by lowering the excretion of uric acid, leading to hyperuricemia (Li et al., 2021).

Clinical guidelines also recommend statins, another group of drugs, as the frontline of treatment for preventing cardiovascular diseases (Cai et al., 2021). Following ST-elevation myocardial infarction STEMI, early high-dosage statin treatment use is linked to immediate and long-lasting clinical improvements. Triglycerides and LDL cholesterol (LDL-C) are linked to adverse changes in the structure and function of the heart. Indeed, it has been shown that lowering LDL-C levels with statin medication lowers the risk of post-STEMI LVR (Bellis et al., 2021). According to a study, atorvastatin treatment dramatically lowered serum uric acid levels in Post Coronary Intervention STEMI patients (Yan et al., 2016).

Aspirin and statins are commonly prescribed together for patients with coronary artery disease. However, the exact role of statins in reducing serum uric acid levels and their interaction with low dose aspirin remains unclear, requiring further investigation. In this study we will investigate the effects of atorvastatin and aspirin alone and in combination on serum uric acid of hyperuricemic rats.

METHODOLOGY

Study Design and Setting

This experimental study was carried out at the Post Graduate Medical Institute (PGMI) in Lahore after the approval of the Institutional Review Committee for Basic Sciences from November 2019 to April 2020.

Sample Size

Total 54 rats, divided into 4 groups of normal rats and 5 groups of hyperuricemic rats, being calculated at 95% confidence interval and 90% power of study by taking anticipated values (mean ± SD) of serum uric acid in placebo and atorvastatin treated hyperuricemic rats (Xilifu et al., 2014).

$$n = \frac{(z_{1-\beta} + z_{1-\frac{\alpha}{2}})^2 (\sigma_1^2 + \sigma_2^2)}{(\mu_1 - \mu_2)^2}$$

$z_{1-\beta}$=1.28

$z_{1-\frac{\alpha}{2}}$= 1.96

μ_1= 216 μmol/l

μ_2 = 62.25 μmol/l

σ_1= 6.15

σ_2= 6.20

Selection of Animals

Inclusion criteria

Sprague Dawley adult male rats of 7-8 weeks age with weight range 150-200g.

Exclusion criteria

Animal with any disease signs.

Sampling Technique

Convenient sampling followed by division into groups by simple lottery method

Experimental Animals

Thirty male Sprague Dawley rats weighing between 150 and 200g and between 7-8 weeks were selected according to inclusion criteria from the PGMI animal house's breeding room. After a week of acclimatization in stainless steel cages, they were divided randomly into five groups of six rats each. Convenient sampling was done, followed by division into groups by simple lottery method.

Study groups

Thirty rats were divided into five groups of six rats each. Group I and II were given distilled water, III, IV and V were given aspirin alone, atorvastatin alone and combination of aspirin and atorvastatin, respectively, as a single morning dose for 7 days. Group I was given normal saline and group II, III, IV and V was given potassium oxonate on day 1, 3 and 7.

ANIMAL GROUPS	DRUG BY ORAL ROUTE	DOSE
I (NORMAL CONTROL)	Distilled Water	5ml/kg
II (DISEASE CONTROL)	Distilled Water	5ml/kg
III (ASPIRIN TREATED)	Aspirin	6.75mg/5ml/kg
IV (ATORVASTATIN TREATED)	Atorvastatin	5mg/5ml/kg
V (ASPIRIN + ATORVASTATIN TREATED)	Aspirin + Atorvastatin	6.75mg + 5mg/5ml/kg

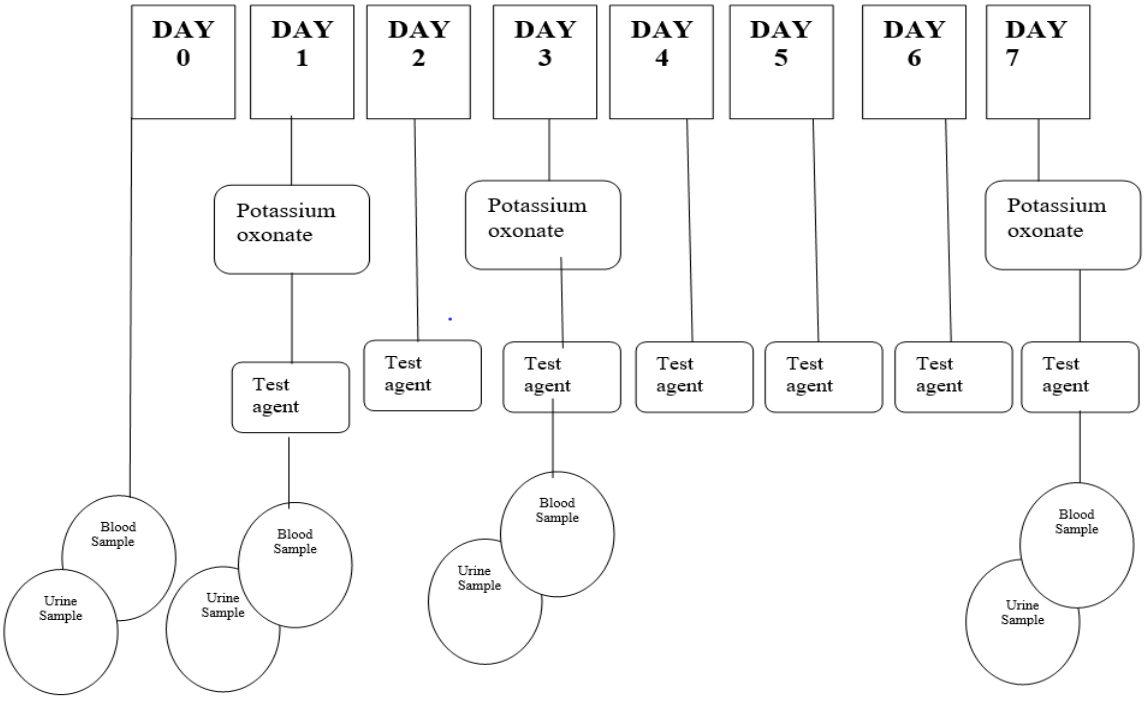
ANIMAL GROUPS	DRUG BY INTRAPERITONEAL ROUTE	DOSE
I (NORMAL CONTROL)	Normal Saline	5ml/kg
II (DISEASE CONTROL)	Potassium Oxonate	250mg/5ml/kg
III (ASPIRIN TREATED)	Potassium Oxonate	250mg/5ml/kg
IV (ATORVASTATIN TREATED)	Potassium Oxonate	250mg/5ml/kg
V (ASPIRIN + ATORVASTATIN TREATED)	Potassium Oxonate	250mg/5ml/kg

Induction of hyperuriucemia

Hyperuricemia was induced by injecting potassium oxonate dissolved in normal saline intraperitoneally in the rats according to 250mg/5ml/kg body weight (Haidari et al, 2008) with the exception of normal control group. It was administered on the 1st, 3rd and 7th days of experiment, one hour before oral administration of test compounds. Numerous research has examined the impact

of pharmacological drugs on uric acid levels using this oxonate-induced hyperuricemic rat model (Kuo et al. 2012; Shah and Shah, 2015).

Parameters



Blood Sample

On day 0(start of the experiment) and on day 7(end of experiment),1 ml blood was withdrawn through cardiac puncture under light anesthesia. At room temperature, the blood could clot in approximately 30 minutes, after that it was centrifuged for 10 minutes at 2500 revolutions per minute. Serum was separated and kept at -20 ºC until quantification of uric acid assay was done (Flores et al., 2020). On the 1st and 3rd days of the experiment, a blood sample was drawn from the rats' tail vein, and a uric acid meter (Multi Sure) was used to measure the uric acid level.

Urine sampling

Urine was collected three hours after the test agent was administered on days 0, 1, 3, and 7 of the experiment. Each rat was kept in a different cage, and the urine was centrifuged for ten minutes at 2500 revolutions per minute. The supernatant was removed and kept at -20°C.

Biochemical Markers

Serum Uric acid

The concentration of uric acid in serum and urine was measured using standard diagnostic kit by enzymatic colorimetric method.

Estimation of creatinine level

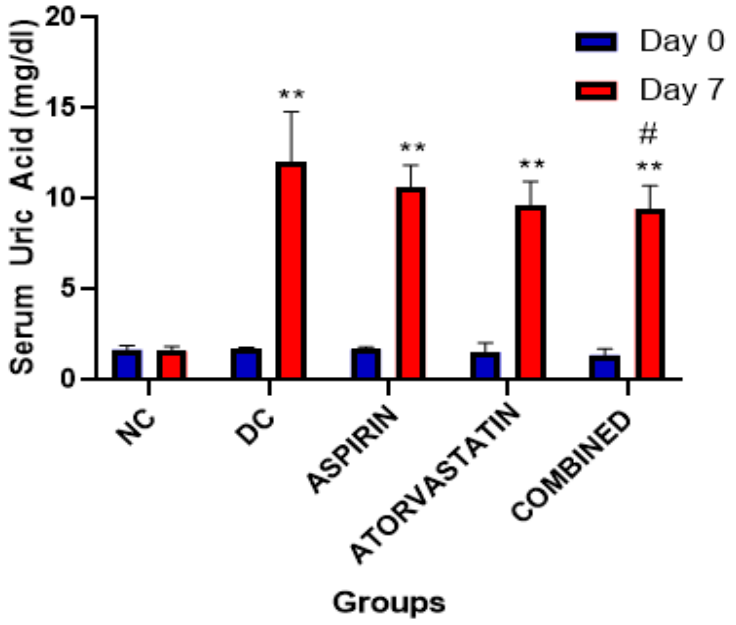
Creatinine levels in serum and urine were measured using commercially available kit by picrate reaction (Jaffe's) method.

Fractional Excretion of Uric Acid

$$FEUA = \frac{serum\ creatinine \times urinary\ uric\ acid}{urinary\ creatinine \times serum\ uric\ acid} \times 100$$

Results

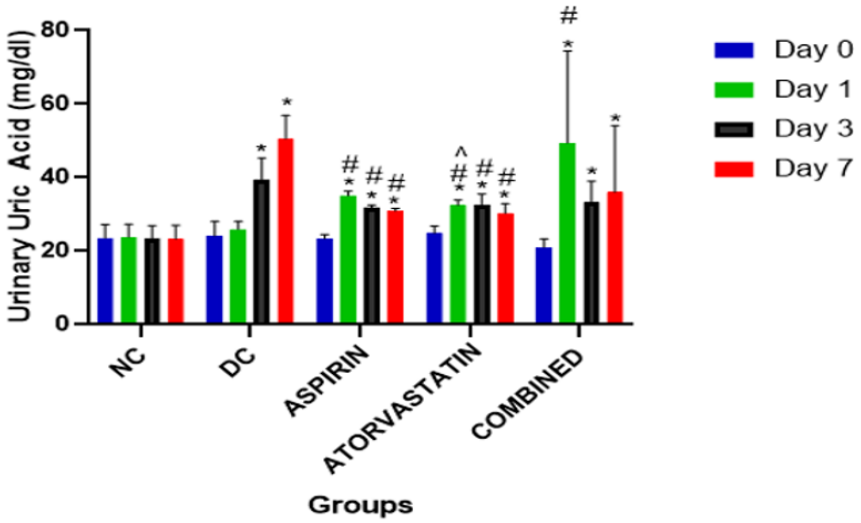
Serum Uric acid



** p value ≤ 0.01 Vs Normal Control

p value ≤ 0.05 Vs Disease Control

Urinary Uric acid

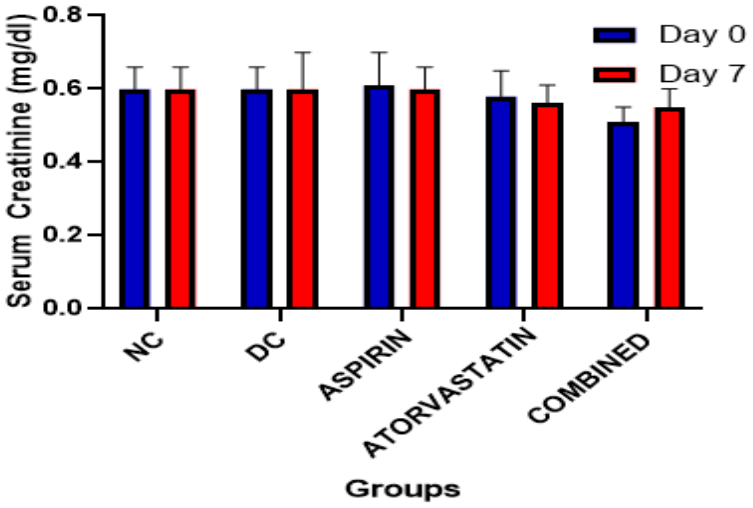


* p value ≤ 0.01 Vs Normal Control

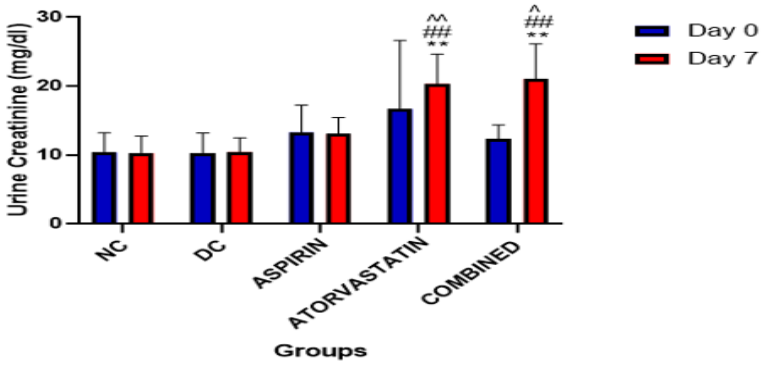
p value ≤ 0.01 Vs Disease Control

^ p value ≤ 0.01 Vs Aspirin Group

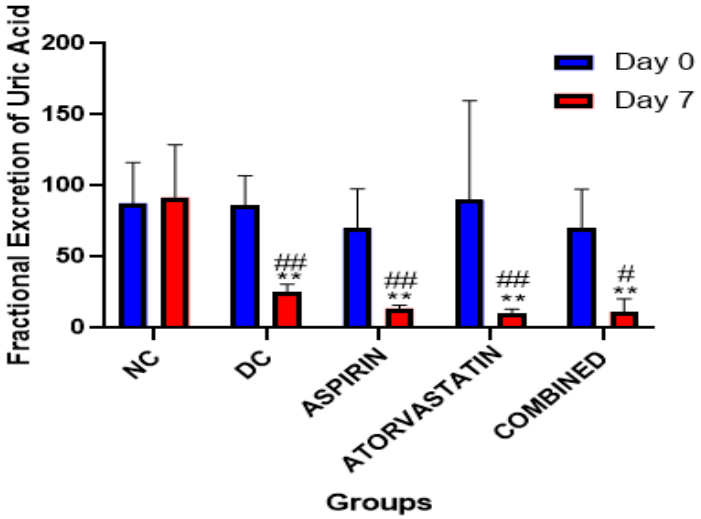
Serum Creatinine



Urine Creatinine



Fractional Excretion of Uric Acid



**** p value ≤ 0.01 Vs Normal Control**

 # p value ≤ 0.05, ## p value ≤ 0.01 Vs Disease Control

Statistical Analysis

SPSS 20 and Graph Pad Prism 5 were used to process the data collected for statistical analysis. The Shapiro Wilk and Kolmogorov Smirnov test was used to assess the data for normality and homogeneity of variance. When it was determined that the data was non normal, a non-parametric statistical test was used for analysis. The numerical data was displayed as graphs and tables with mean ± SD and median (interquartile range). Kruskal-Willis test was used to determine the difference of results among all groups. To identify differences between individual groups, the Mann-Whitney U test was used. The significance of the results over time within each group was determined using the Wilcoxon signed-rank test. A p value of ≤ 0.05 was considered statistically significant, ≤ 0.01 highly significant and ≤ 0.001 very highly significant.

DISCUSSION

Patients with atherosclerotic cardiovascular diseases are frequently prescribed low dose aspirin as antiplatelet therapy to lower the risk of adverse health outcomes (Jones et al., 2021). Low doses of cardio protective aspirin have an anti-uricosuric effect, it has been found to trans-stimulate URAT 1, increasing its activity and leading to enhanced uric acid reabsorption (Ragab et al., 2017). This effect predisposes normal individuals to develop hyperuricemia and aggravation of gout in patients already suffering from raised uric acid levels (Pawar et al., 2017). Additionally, elevated uric acid levels may be directly linked to a higher risk of dying from cardiovascular events (Bakris et al., 2014). Statins are used to treat elevated LDL cholesterol because they are competitive inhibitors of the HMG-CoA reductase enzyme. Apart from their lipid-lowering property, statins are also thought to decrease serum uric acid and raise nitrite levels, which helps reducing the risk of cardiovascular diseases.(Xilifu et al., 2014). Thus, it is proposed that statins can prevent aspirin-induced hyperuricemia, and that aspirin and statins may have antagonistic effects when taken together.

The objective of this study was to make a hyperuricemic rat model using potassium oxonate and to examine the effects of low doses of atorvastatin, aspirin, and both on the concentration of uric acid in the serum and the excretion of uric acid in the urine of hyperuricemic rats.

In this study, 250 mg/kg potassium oxonate was injected intraperitoneally into all groups for the induction of hyperuricemia, on day 1, 3, and 7. As a result, a successful hyperuricemic rat model was developed, and the blood uric acid levels of the disease control and normal control showed a statistically significant difference ($p = 0.004$). These findings were consistent with those of Shah and Shah (2015), who used similar models to induce hyperuricemia.

To examine how atorvastatin and aspirin, alone and in combination, affect uric acid levels in rats with hyperuricemia, thirty rats were divided into five groups of six rats each. Group I and II were given distilled water orally as a single dose in the morning for seven consecutive days. Group III, IV and V were given aspirin alone, atorvastatin alone and aspirin and atorvastatin in combination, orally as a single dose in morning for seven consecutive days. On day 1st, 3rd and 7^{th} day, potassium oxonate was administered intraperitoneally to all the groups to induce hyperuricemia. Blood and urine samples were collected on day 0, 1, 3 and 7. Serum uric acid, serum creatinine, urinary uric acid, urine creatinine and fractional excretion of uric acid were estimated.

Aspirin raised serum uric acid levels in both normal and hyperuricemic rats (p values 0.004 and 0.006 vs. normal control, respectively). Similar findings were made by Thomson et al. (2013), who found that aspirin considerably increased serum uric acid levels when compared to normal control. In comparison to the normal and disease control, atorvastatin alone did not significantly lower the uric acid levels of hyperuricemic rats. This runs counter to a human study by Abuel Hassan et al. (2018), which found that atorvastatin significantly reduced blood uric acid in hyperlipidemic patients with

normal baseline serum UA when compared to matched controls. The current study's findings are contradicted by other human studies that have shown that atorvastatin lowers serum uric acid levels in patients with hyperuricemia (Athyros et al., 2007; Kose et al., 2014; Yan et al., 2016).

Although aspirin+atorvastatin reduced blood uric acid levels in hyperuricemic rats as compared to disease control (p value 0.030), uric acid levels were still considerably higher than those of normal control (p value 0.004).

When compared to the disease control, aspirin and atorvastatin considerably reduced the amounts of uric acid in the urine of hyperuricemic rats. Nevertheless, other investigations found that low-dose aspirin may increase SUA by lowering the excretion of uric acid, leading to hyperuricemia (Li et al., 2021). This is in contrast to the research done by Millionis et al. (2004) and Ogata et al. (2010), which found that atorvastatin decreases uric acid reabsorption from proximal tubules and increases glomerular filtration, which results in a hypouricemic impact in hyperlipidemic patients.

In both normal and hyperuricemic rats, aspirin and atorvastatin, both separately and together, did not significantly alter serum creatinine levels. This study is in accordance with the studies by Louthrenoo et al. (2002) and Millionos et al. (2004), which found that serum creatinine did not change during or after aspirin and atorvastatin treatment, respectively. When compared to the normal control and disease control, atorvastatin alone and in combination with aspirin increased urine creatinine in hyperuricemic rats (p values 0.004, 0.006, 0.010, and 0.010, respectively). These findings are consistent with the research conducted by Srinivas et al. (2008), who found that atorvastatin raised urine creatinine ($p < 0.05$) in rats with atherosclerosis.

CONCLUSION

The results could not affirm the hypothesis of nullifying the hyperuricemia effect of low dose aspirin with the addition of atorvastatin in the hyperuricemic rats.

Comparing hyperuricemic rats to the normal and disease control groups, atorvastatin also reduced the fractional excretion of uric acid (p values 0.004 and 0.006, respectively). This is in contrary to the findings of Milionos et al. (2004), who found that FEUA dramatically rose in hyperlipidemic patients following a 6-week course of atorvastatin medication. Urinary creatinine may have increased as a result of atorvastatin's decrease in fractional excretion of uric acid in the current study. It is also interesting that normal rats treated with atorvastatin for four weeks in the current study did not see a significant change in FEUA.

Limitations

The study's limitations include the estimation of just serum uric acid and urine uric acid excretion. A more thorough impact on oxidative stress markers and renal parameters would have improved data interpretation and yielded valuable information. Similarly, taking atorvastatin just once might not be enough to provide meaningful effects. However, atorvastatin's function in uric acid elimination alone is insufficient to reverse the effects of aspirin-induced hyperuricemia in rats, according to the study's available data.

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