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Original Article

Allopurinol therapy impairs lipid metabolism in patients with renal stone

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ABSTRACT

Majority of the cardiovascular diseases have many predisposing and aggravating factors that cause atherosclerotic vascular injuries. Uric acid has been reported as a contributory factor that negatively interfere with the pathology of cardiovascular diseases and may even induce cerebrovascular complications. Allopurinol is the commonly used uricosuric agent in patients with gouty superimposed by urate renal stone. The present study aimed to identify the effect of allopurinol on the serum lipid parameters as an inevitable complication in patients diagnosed with renal stone. Fifteen patients diagnosed with urate renal stones were treated by allopurinol (300 mg/day for 6 months) and blood samples were obtained for laboratory assessment of the lipid profile. The outcome showed significant alterations of lipid parameters (with elevated total cholesterol (TC) and triglycerides (TG), plus reduced HDL serum levels); hence LDL, VLDL, and non-HDL were elevated accordingly. The study led to the conclusion that using allopurinol in subjects with apparently normal lipid parameters was associated with elevation of the predisposing factors of cardiovascular diseases. These findings give a general clue and might be helpful in selection of appropriate uricosuric agents for treatment of patients with cardiovascular diseases, suggesting that, the avoidance of allopurinol is advisable in predisposing high risk groups of patients, and necessitating the searching for safe-alternative hypouricemic agents in such group.

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KEYWORDS: allopurinol, lipid, uric acid, renal stone, hyperuricemia.

INTRODUCTION

There are accumulating evidences about the link between hyperuricemia and the lifestyle-based diseases, such as hyperlipidaemia, hypertension, atherosclerosis, and coronary arterial diseases [1-3]. Low-density lipoprotein (LDL) is a direct risk factor for cardiovascular diseases, and LDL reduction is considered as the main pharmacological approach for treatment of cardiovascular diseases. Therefore, determination of possible risk factors of LDL elevation is of great clinical relevance. Epidemiological studies have identified that uric acid is greatly associated with LDL elevation and cardiovascular diseases [4, 5]. Moreover, cross-sectional studies have reported an association between hyperuricemia and high plasma triglyceride (TG) and high-density lipoprotein (HDL) level [6-8]. Allopurinol has been considered as a gold-standard therapy for treatment of gout and hyperuricemia [9]. However, many adverse effects have been reported with allopurinol use, including hepatic and renal dysfunction, vasculitis and Stevenson-Johnson syndrome [10].

Xanthine oxido-reductase irreversibly catalyzes the conversion of xanthine to uric acid, and this step is a rate-limiting step in purine pathway and the outcome is reciprocal to elimination through kidney or catabolism in liver [11, 12]. It has been confirmed that xanthine oxido-reductase enzyme is involved in the metabolism of glucose and lipid. One of the main involvements of xanthine-metabolic pathways in lipid peroxidation is the

production of superoxide free radical [13, 14]. Superoxide has been considered as a major contributor to lipid peroxidation under ischemic condition. Lipid peroxidation associated with xanthine-metabolic pathway increases the chance of developing atherosclerosis and myocardial dysfunction [15, 16]. In normal physiological conditions, xanthine oxidase (in dehydrogenase form) accepts an electron from adenine dinucleotide. However, under pathological conditions of ischemia, the dehydrogenase form is oxidized into xanthine oxidase and accumulated in tissues resulting in more conversion of purine to uric acids. Xanthine oxidase is also produced in pathological tissues as a byproduct of inflammatory reactions involving phagocytes (neutrophils and macrophages). These inflammatory reactions participate in further lipid peroxidation resulting in the progression of the pathological conditions [17].

Structurally, xanthine-oxidoreductase is homodimeric with three domains: a molybdenum-containing molybdopterin cofactor (Moco), C-terminal, and flavin adenine dinucleotide (FAD) cofactor alongside the redox system which consists of iron and sulphur. Collectively, these factors and elements participate together for maintaining electron transfer (see Figure 1). The molecular mechanism of action of this enzyme eventually produce uric acid, NADH, superoxide, hydrogen peroxide, and other free radicals through complicated steps which collectively have a deleterious impact on various metabolic pathways [18-20].

High level of LDL is a direct contributory factor for vascular diseases [1]. A compiled report indicated that there is a complex relationship between the level of uric acid and lipid biomolecules [2]. Recent epidemiological studies have indicated that the solid interaction between hyperuricemia and some metabolic biomarkers like fasting blood glucose and lipid profile predispose the affected patient to metabolic syndrome [21, 22] together with precipitation of urate-renal stone (Figure 2) [12, 23]. Another cross-sectional study has confirmed that hyperuricemia is a direct predisposing factor for elevated triglycerides (TG) level and reduced HDL level [24, 25]. Investigators have increasingly reported that hyperuricaemia is a direct cause of lipid dysmetabolism and consequently metabolic

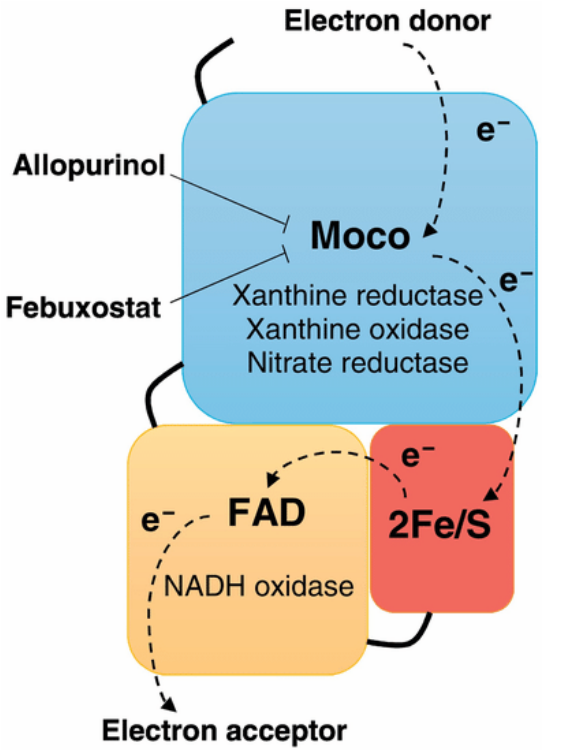

Figure 1. Xanthine oxidation-reduction reaction and molecular mechanism of inhibitors. Allopurinol and febuxostat inhibit the enzyme through a complicated mechanism involving electron transfer through flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD+).

syndrome and they had confirmed this through the fructose test (Figure 3). This test confirms that hyperuricemia modulates hepatic fructose catabolism in fructose-high diet laboratory animals with end results of higher ATP production and increased purine catabolism to uric acids [26].

Allopurinol is a xanthine oxidase inhibitor and is indicated for hyperuricemia-associated diseases [27]. Allopurinol has been shown to interfere with lipid peroxidation and modulate metabolic activity in patients with metabolic syndrome [28]. However, there is a controversy regarding the results achieved from allopurinol-associated lipid dysmetabolism; conflicting results might be due to variation in the samples included in the study and the fact that some patients have other diseases, such as, diabetes, hyperlipidaemia, atherosclerosis, cardiovascular diseases, and metabolic syndrome. The present study was designed to study the effect of allopurinol on lipid metabolism and involved apparently healthy

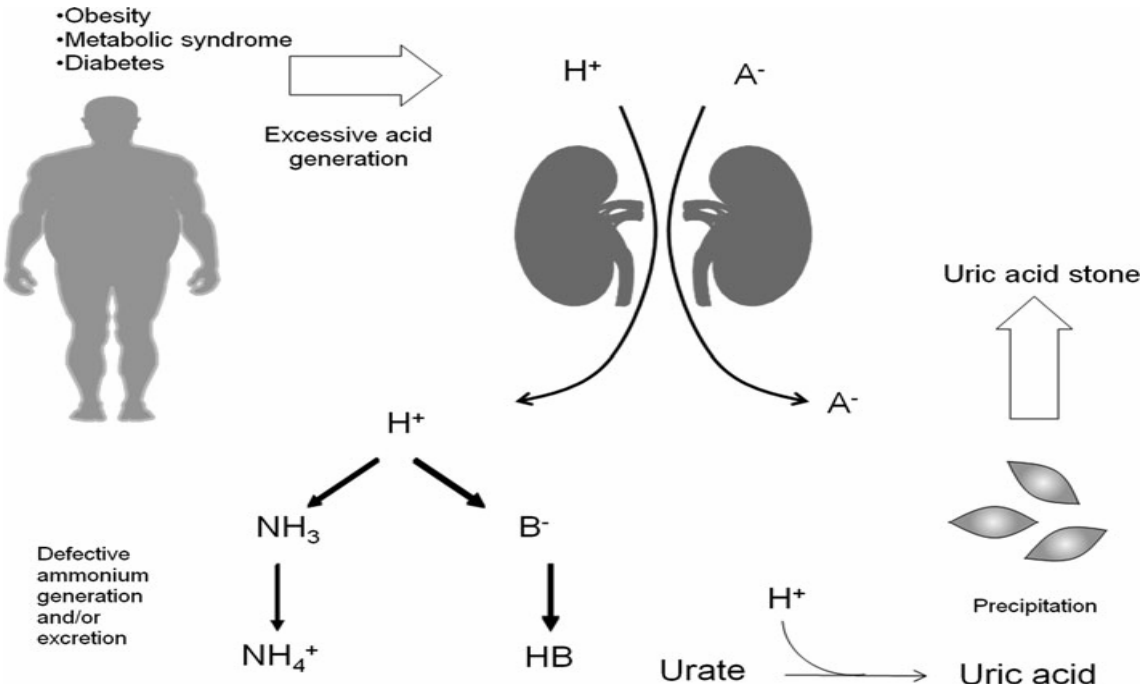

Figure 2. Factors predisposing to metabolic syndrome and renal stone in hyperuricemic patients.

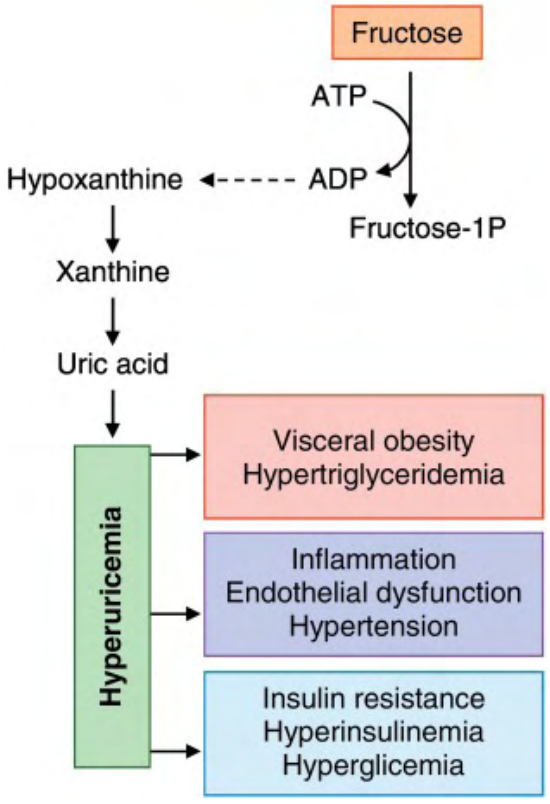

Figure 3. Fructose test and hyperuricemia.

subjects with urate-renal stone and no association of systemic diseases.

MATERIALS AND METHODS

A total of 15 clinically treated patients with renal stone (9 male and 6 female) were enrolled in this study and were treated with allopurinol (300 mg/day) for a period of 6 months. The demographic parameters of the patients are mentioned in the Table 1 below. A consent form of agreement was taken from all participants. The exclusion criteria comprised a history of any chronic diseases (including, thyroid disease, hypertension, angina pectoris, atherosclerosis, myocardial infarction, heart failure, renal or hepatic insufficiency) or those taking any chronic medication. Pregnant women and lactating mother were also excluded from the study.

For laboratory analysis of biochemical parameters, six milliliters of venous blood was withdrawn from overnight fasting patients before and after 6 months of allopurinol therapy. The blood was left at room temperature to clot and after centrifugation the serum was separated in plane tube, and samples were collected and stored in freezers (-20 °C) to

Table 1. Biochemical and demographic characteristics of the studied groups.

	Allopurinol Therapy (n = 15)	
Parameters	**Before**	**After**
Age (years)	39.41 ± 3.8	-------
BMI (kg/m^2)	28.2 ± 3	28.5 ± 2.8
Duration of treatment	6 months	-------
Gender (male/Female)	9/6	-------
Uric acid	6.25 ± 0.7	5.64 ± 0.65*
Albumin	4.13 ± 0.5	4.17 ± 0.61

*P<0.05, BMI = body mass index, kg = kilogram, m^2 = square meter

be used for analysis. Frozen samples were thawed and analyzed. Enzymatic method (Cobas 6000, Roche-Diagnostic) was used for measuring fasting serum total cholesterol (TC), TG, and HDL-c. The principle of assay was based on the enzymatic reaction of previously prepared plasma sample.

The plasma samples were previously treated with magnesium and phosphotungstic acid to precipitate plasma components, except the measured cholesterol associated with lipoprotein, followed by an enzymatic step resulting in the formation of formazan dye, the intensity of which correlates proportionally with the concentration of cholesterol. Similarly, TG was measured based on the intensity of formazan dye produced from hydrolysis of TG into glycerol and fatty acids using lipoprotein lipase enzyme. LDL cholesterol is calculated using the Friedewald formula (LDL = TC - HDL – TG/5) [29]. The Cobas 6000 instrument calculates the vLDL cholesterol (TG/5 ratio) as well as the non-HDL cholesterol (TC- HDL) from the measured values.

For cholesterol measurement, the plasma samples were first diluted with phosphate buffer solution, and the cholesterol ester in the samples were de-esterified to cholesterol and free fatty acids. In the presence of cholesterol dehydrogenase, nicotinamide-adenine dinucleotide (NAD) oxidizes cholesterol in the samples to cholestenone and NADH. The resulting cholestenone were then reduced to formazan dye in the presence of diaphorase enzyme and NADH through consecutive steps of oxidation and reduction reactions. The color intensity of formazan is proportional to cholesterol concentration. For triglyceride measurement, the triglycerides in the samples were hydrolyzed to glycerol and free fatty acids *via* lipoprotein lipase. In the presence of glycerol dehydrogenase, glycerol and NAD produce dihydroxyacetone and NADH. These end-products were converted into formazan dye *via* diaphorase enzyme supplied by the manufacturer; the optical density was determined at 460 nm. The color intensity of formazan dye is directly proportional to the level of triglycerides in the sample.

Additionally, albumin was measured before and after treatment with allopurinol to confirm normal functioning of liver and to exclude the renal failure which might be associated with renal stone formation. The level of albumin before and after allopurinol was within the normal range (see Table 1). Albumin was measured by ELISA technique (Human Albumin ELISA Kit (abcam). The assay was based on loading plasma samples and standard on 96-wellplates. The plate was pre-coated overnight with capture antibody specific to albumin, and on the next day blocked for 1 hour with 1% bovine serum albumin buffer. Subsequently, the wells were exposed to albumin-specific detection antibodies. Each single step was followed by discarding the plate contents forcibly and washing (four times) by diluted washing buffer. The preparation of washing buffer involves mixing tween-20 detergent with PBS at a concentration of 0.05%. Finally an enzymatic reaction was initiated by adding Avidin-Biotin-Peroxidase Complex; this reaction was visualized by a chromogenic reagent provided with the kit resulting in blue color formation and was finally terminated by stop solution producing yellow color, the intensity of which is reciprocal to the albumin concentration in the test sample. Each individual step was followed

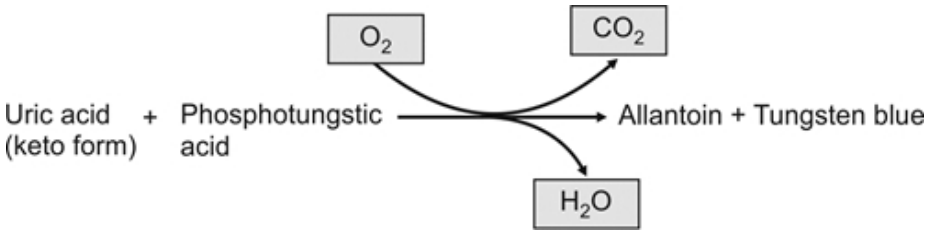

Figure 4. Principle based on measurement of uric acid.

by washing the wells with washing buffer. A visible signal was determined using a plate reader at 405 nm and the concentration of samples was calculated *via* interpolation of standard calibration curve.

In order to confirm that allopurinol drug reduces uric acid levels, we measured the uric acid levels before and after initiation of the therapy (see Table 1). Uric acid was measured by colorimetric method. According to manufacturer instruction, the standard and plasma samples were treated with the reaction mix and incubated for a period of 30 minutes and measured then by microplate reader and analyzed according to manufacturer instruction. The principle of the reaction is based on oxidation of uric acid to allantoin and CO_2 by phosphotungistic acid reagent in alkaline solution followed by reduction of the resulting phosphotungistate to tungsten blue which was measured spectrophotometrically at the wavelength of 710 nm. The proteins in the samples are removed by precipitation with trichloroacetic acid (see Figure 4).

Data were expressed as the mean ± standard deviation. Comparisons between the investigated parameters for allopurinol- treated patients before and after therapy were conducted using the t-test. $P < 0.05$ was considered a statistically significant difference. Statistical results were obtained using GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA). The histogram chart was designed using a Microsoft Office 2010 Excel program.

RESULTS AND DISCUSSION

The present study demonstrated that chronic use of allopurinol is associated with elevation of serum level of lipid parameters in subjects with normal plasma baseline levels of TC and TG together with reduction of HDL compared to normal baseline (Figure 5). This deleterious impact of allopurinol imparts that a guidance and care should be taken toward the application of such medication in patients with cerebrovascular and cardiovascular ailments. It has been well documented that uric acid has a contributory role in elevated lipid parameters; however, lowering the serum uric acid by hypouricaemic agents should enhance lipid parameters regardless of patients' status and demographic characteristics. Conversely, the present study demonstrated that long-term use of allopurinol has a negative impact on lipid parameters.

The primary finding of this study is elevation of LDL after chronic allopurinol therapy (Figure 6). Calculated concentrations of LDL were significantly ($p < 0.001$) elevated after (116 ± 22 mg/dl) allopurinol therapy compared to (50 ± 7 mg/dl) prior to the start of allopurinol therapy. The role of uric acid in the regulation of lipid metabolism is incompletely understood; however, suppression of lipid peroxidase [30], and inhibition of lipoprotein lipase are well-known processes [31]. Many studies have reported a positive correlation between elevated uric acid and plasma lipid parameters; for instance, a study conducted by Shelmadine *et al*. (2012) concluded that uric acid and LDL were directly linked to each other in metabolic syndrome patients [32]. The mechanism might be due to the activity of lipoprotein lipase (LPL), the enzyme which is responsible for TG and LDC metabolism [33]. Reduction of LPL has been shown to be correlated to plasma uric acid level [32, 34]. The reduction in LPL has a significant effect on oxidative stress and decreased nitrous oxide production which might be associated with elevated LDL and TG which is reported in the present study [33]. Moreover, oxidative stress might be associated with impaired TG level due to accumulated oxidized LDL [33]. In contrast to our results, prospective studies using allopurinol carried

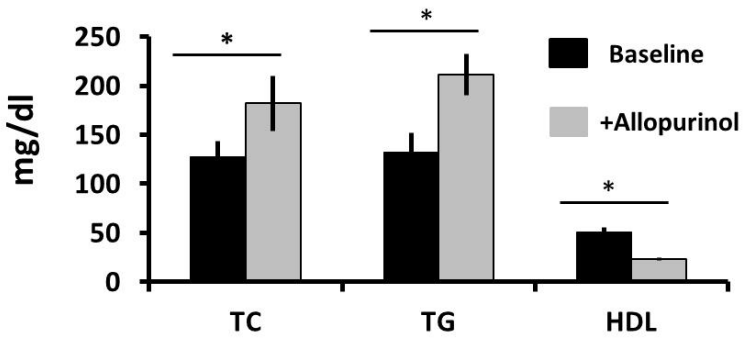

Figure 5. Allopurinol impaired lipid metabolism. 6 month use of allopurinol increased TC, TG, and increased HDL levels as compared to the same subjects' baseline levels; data expressed as mean ± SD, *p < 0.001. TC = serum total cholesterol, TG = serum triglyceride, HDL = high density lipoprotein.

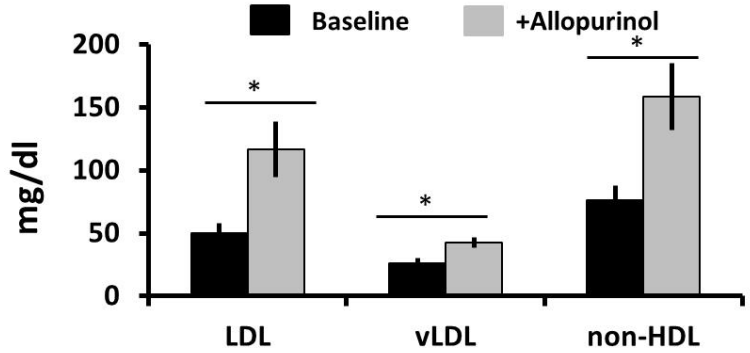

Figure 6. Allopurinol impaired calculated lipid parameters. 6 months' use of allopurinol increased LDL, vLDL, and non-HDL levels, as compared to the same subjects' baseline levels; data expressed as mean ± SD, *p<0.001. LDL = Low density lipoprotein, vLDL = very low density lipoprotein, non-HDL = non-high density lipoprotein.

out by Perez-Pozo *et al.* [35] and Shelmadine *et al.* [32] reported a direct relationship between uric acid and LDL. Moreover such relationship has been further investigated by preclinical [32, 36] and clinical [37-39] studies. The subjects involved in the present study were normal with regard to plasma baseline lipid levels and this should be taken into account while comparing with other studies.

A clinical study conducted by Wu *et al.* (2019) [39], comparing hypolipidaemic effects of different urate-lowering drugs has concluded that all of the used urate-lowering therapy reduced plasma triglycerides and cholesterol levels; however, the reduction was the most with febuxostat and lowest with allopurinol next to benzpromarone. This finding contradicts with our results and stated that further studies should be accomplished to confirm the hypolipaedimic efficacy of febuxostat as a safer alternative replacing allopurinol.

Another finding of the current study is reduction of HDL after chronic allopurinol therapy compared to baseline level. The concentrations of HDL were significantly ($p < 0.001$) reduced after (23 ± 1.4 mg/dl) allopurinol therapy compared to (51 ± 4.4 mg/dl) prior to allopurinol therapy (Figure 6). A preclinical laboratory study on laboratory rats exposed to different concentrations of TC-containing diet concluded that allopurinol has a positive impact on blood pressure parameters with no changes reported in HDL levels [28]. Correspondingly, the same conclusion was achieved by a long-term randomised controlled clinical trial based on comparing allopurinol-treated group versus control group taking into consideration that the enrolled subjects were diabetic and were treated by hypo-lipidaemic agents [29]. To further confirm these findings, vLDL and non-HDL levels were calculated and the results of the calculation showed elevated

vLDL and non-HDL biomolecules (Figure 6). Plasma non-HDL represents a total value of all atherogenic apoB- lipoproteins: vLDL, IDL, LDL, chylomicron remnants and Lp (a) while vLDL mainly corresponds to the plasma TG level [40].

This prospective study suggests that allopurinol has a negative impact on lipid parameters in patients with renal stone who have no other associated diseases and have normal laboratory parameters for lipid plasma baseline levels. These findings further confirm the current available knowledge and further complicate the controversy regarding allopurinol role in lipid metabolism. However, the main limitation associated with the present study is the small sample size, young-age group of users, normal lipid baseline levels, and non-obese subjects (Table 1). These confounding factors are not representative of the general population of allopurinol users and therefore conducting large clinical trials involving more subjects of different age groups with different body mass index is very much required.

CONCLUSION

The deleterious impact of allopurinol on various biochemical parameters was studied extensively; however, there is a controversy regarding the impact of allopurinol on lipid parameters. The present study confirmed that allopurinol impairs lipid metabolism by increasing serum level of TC and TG, together with reducing HDL. Therefore, the beneficial effect of allopurinol on the uric acid reduction as a potential mediator of cardiovascular disease could be antagonised by the impaired level of lipid dysmetabolism, suggesting that allopurinol medication may be indicated cautiously in patients with cardiovascular ailments and that a search for alternative hypouricaemic agents in such cases might be needed.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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