Ameliorative Effects of Lesinurad (Zurampic) on Experimental Hyperuricemia, Biochemical, Molecular and Immunohistochemical Study

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Abstract

Background: The current study evaluates the potential ameliorative impact of Lesinurad (i.e. Zurampic (ZUR)) and Allopurinol (ALP) on the kidneys of hyperuricemic mice at the biochemical, molecular and cellular levels.

Methods: ALP and ZUR in combination were orally administered to both hyperuricemic and control mice for seven consecutive days. Levels of uric acid and Blood Urea Nitrogen (BUN), along with antioxidants and inflammatory cytokines (IL-1β and TNF-a) were measured in serum. The mRNA expression of mouse urate anion transporter-1 (mURAT1), glucose transporter 9 (mGLUT9), organic anion transporters (mOAT1 and mOAT3), in renal tissues were examined using quantitative real time PCR (qRT-PCR). Simultaneously, the immunoreactivity of transforming growth factor-1 beta (TGF-β1) was examined immunohistochemically.

Results: ALP and ZUR administration resulted in significantly reduced serum urate levels and decreased serum levels of uric acid, BUN, catalase, glutathione peroxidase (GPx) and inflammatory cytokines (IL-1β and TNF-a) in hyperuricemic mice. Both partially reversed oxonate-induced alterations in renal mURAT-1, mGLUT-9, mOAT-1 and mOAT-3 expressions, as well as leading to changes in the immunoreactivity of TGF- β1, resulting in the increase of renal uric acid secretion and excretion. The combined administration of ALP and ZUR restored all altered measurements in a synergistic manner, improving renal dysfunction in the hyperuricemic mouse model employed.

Conclusion: This study therefore provides evidence for the synergistic hypouricemic impact of both ALP and ZUR in the treatment of HU in mice at the biochemical, molecular and cellular levels.

Background

Uric acid (UA) is the end product of an exogenous and endogenous pool of purines metabolism. The exogenous pool is dependent on diet, with a significant contribution from animal proteins. The endogenous pool depends on the production of uric acid from the liver, intestines and other tissues [1]. Potential sources of the exogenous pool of HU consists of food (i.e. purine rich products), glucose, and fructose [2], which it is controlled by xanthine oxidase, which irreversibly oxidizes xanthine into UA [3, 4].

UA is primarily excreted through the kidneys in urine (65–75%) and to a lesser extent through the gastrointestinal tract (25–35%) [3, 5]. An increase in the level of uric acid in the blood is known as Hyperuricemia (HU), and is the cause of gout, which manifests in inflammatory arthritis and painful disabling with acute attacks [6].

HU is defined as an increase in UA levels over 7 mg/dL in men and 6 mg/dL in women [7]. As well as obesity and hypertension, HU is mainly associated with the following: (1) alcohol consumption; (2) a fructose rich diet; (3) excess consumption of seafood or meat; (4) diuretics; (5) some medications; and (6) angiotensin converting enzymes [8, 9]. Gout is associated with precipitation of Monosodium Urate

(MSU) crystals in joints and soft tissues [10, 11]. Deposition of MSU crystals in the big toe, some joints and the ankle are associated with neutrophil infiltration, swelling and pain [12]. The first line of gout treatment is allopurinol, an xanthine oxidase inhibitor stimulating the renal secretion and excretion of UA [11]. Other anti-inflammatory non-steroidal drugs known to inhibit cyclooxygenase activity (i.e. cortisol, indomethacin and glucocorticoids) are beneficial for the treatment of gout [13]. However, these medications have severe side effects and drug–drug interactions capable of harming human health [14].

A promising approach for the treatment of HU and its associated complications consists of alternative therapies, i.e. dietary flavonoids and hypouricemic curative agents with suboptimal doses devoid of undesirable Allopurinol (ALP) side effects. The need to identify safe drugs is therefore vital for both physicians and patients. A new medication used for treatment of HU is Lesinurad, commonly named Zurampic (ZUR). ZUR was approved in December 2015 by the US Food and Drug Administration and works on the urate-anion exchanger transporter (URAT1) in combination with ALP [15]. Furthermore, URAT1 is a trans-membrane protein that acts as a urate-specific and organic anion exchanger, being localized in the luminal membrane of the proximal convoluted tubules [16]. Urate is filtered in the glomerulus and reabsorbed in the proximal convoluted tubules through the influence of URAT1. ZUR inhibits both the URAT1 and Organic Anion Transporters (OATs) controlling uric acid reabsorption, while at the same time increasing the urinary excretion of uric acid [17].

Lesinurad induces significant urinary excretion of uric acid. A previous study postulate that the declined levels of serum uric acid levels take place in response to the influence of ZUR on renal urate. The current study therefore examines the ameliorative impact of Lesinurad and ALP in combination on oxonate-induced HU in mice at the biochemical, molecular and histopathological levels.

Materials And Methods

Chemicals and Kits

Potassium Oxonate (PO), agarose, ALP and ethidium bromide were purchased from Sigma-Aldrich (St. Louis, MO, USA). ZUR was purchased from Ironwood Pharmaceuticals, Inc., Cambridge, MA02142. 100 bp DNA ladder and reverse transcriptase enzymes were from MBI (Fermentas, Thermo Fisher Scientific, USA). Qiazol and Oligo dT were from QIAGEN (Valencia, CA, USA). The kits for catalase, MDA and Glutathione Peroxidase (GPx) were purchased from Biodiagnostic Co. (Dokki, Giza, Egypt). While the kits of GPT, GOT, BUN and uric acid were from BIO-MED Diagnostics and EGY- CHEM for lab technology, Badr City, Industrial Area Piece 170, 250 Fadan In East of Elrubaki, Egypt. Xanthine Oxidase kit (Catalog No: E-BC-K024), mouse IL-1 beta (Catalog No: E-EL-M0037) and mouse TNF-alpha (Catalog NO: E-EL-M0049) were obtained from Elabscience Biotechnology Inc. USA.

Experimental Animals and Design

The Scientific Deans of Taif University, Saudi Arabia, along with its Ethical Committee, approved all procedures and in vivo animal usage in this study for the project #1–439–6099, based on the NIH Guide for the Care and Use of Laboratory Animal. The Swiss mice used in this experiment consisted of a total of forty-two male mice aged ten weeks (weight 30-35g), originating from the College of Pharmacy, King Abdel-Aziz University, Jeddah, Saudi Arabia. The mice were handled manually for seven days in order to overcome stress. The animals were kept in a 12/12 hrs day-dark cycle and were given free access to food and water. Seven groups of mice were employed, each consisting of six individuals.

Group 1 was the negative control (CNT), with the mice given free access to food and water. Group 2 was the positive hyperuricemic group, in which the mice received PO intraperitoneally (250 mg/kg bw, single dose every day at 8:00 am). The dosages of PO and timing were determined as stated above [36]. Group 3 received ALP orally (ALP; 5 mg/kg body weight daily one hour after PO administration) for seven days [18]. Group 4 was administered ZUR, accompanied by an orally administered dose of 80 mg/kg, in accordance with Wu et al. (2017) [37]. For seven days, groups 5 and 6 were administered PO at 8.00 am followed by ALP for Group 5 and ZUR for Group 6 one hour later (9.am). For seven consecutive days, Group 7 was administered PO first at 8:00 am, followed by a combination of ALP and ZUR at 9:00 am. After two weeks and at the end of the design, the mice inhaled dimethyl ether and were decapitated after overnight fasting. Serum was extracted and stored at –20 $^{\circ}$C until the chemistry measurements took place. Kidney and liver tissues were soaked in Qiazol for RNA extraction and in Bowan's solution for histology and cellular immunohistochemistry.

Serum biochemistry, antioxidants, and cytokines assessments

The serum levels of the antioxidants, liver and kidney biomarkers and cytokines were assayed calorimetrically, using specific commercial kits based on the manufacturer's instruction manual.

Xanthine Oxidase activity

The kit was used for the determination of XOD activity in serum, plasma, erythrocyte and tissue. XOD catalyzes hypoxanthine, to form xanthine and superoxide anion free radicals, resulting in a purplish red substance in the presence of electronic receptors and a chromogenic agent. XOD activity can be calculated by measuring the OD value of the purplish red substance at 530 nm. For liver tissues, following homogenization in normal saline on ice, in a ratio of one liver tissue and nine for normal saline, homogenate is centrifuged for ten minutes and supernatant used for assay. The XOD unites for serum values are U/l, and for liver homogenates are U/g protein tissue. The protocol employed was partially modified in accordance with the method of Haidari et al. (2011) [38].

RNA extraction, cDNA synthesis and quantitative real time PCR (qRT-PCR)

Total RNA was, as described elsewhere [39], extracted from the kidney tissues and frozen samples homogenized in a homogenizer. Chloroform (200 µL) was added to the homogenate, then centrifuged at 4 °C at 12000 rpm for ten minutes. The supernatant was separated and an equal volume of isopropyl alcohol was added. The pellets of RNA were dissolved in Diethylpyrocarbonate (DEPC) water after washing and drying. The integrity of RNA was confirmed [40] and 3 µg of total RNA and 0.5 ng oligo dT (Qiagen Valencia, CA, USA) was denatured following incubation in a Bio-Rad T100^{TM} Thermal Cycle at 70 °C for five minutes. Denatured RNA was reverse transcribed after the addition of 2 µL of 10 mM dNTPs, 100 U of M-MuLV (SibEnzyme, Ak, Novosibirsk, Russia) and 2 µL of 10X RT-buffer, before being incubated in a Bio-Rad T100^{TM} Thermal Cycler for one hour at 37 °C, then for ten minutes at 90 °C, to ensure enzyme inactivation. For quantitative real time PCR analysis (qRT-PCR), primers for the examined genes (Table 1) were designed using GenScript Real-time PCR (TaqMan) Primer Design (https://www.genscript.com/tools/real-time-pcr-taqman-primer-design-tool). Each PCR reaction consisted of 1.5 µl of 1µg/µl cDNA, 10 µl SYBR Green PCR Master Mix (Quanti Tect SYBR Green PCR Kit, Qiagen), along with 1 µM of forward and reverse primer for each examined gene and nuclease free H_2O to a final volume of 20 µl. Reactions were run and analysed in Applied Biosystem 7500 Fast Real time PCR Detection system. qRT-PCR conditions are: 95°C for ten minutes (first denaturation) and forty cycles of 95°C for fifteen seconds (second denaturation stage) followed by 60°C for one minute (annealing and extension stage). The critical threshold (Ct) of the target gene was normalized with quantities (Ct) of the housekeeping gene (β-actin), using the formula x = $^{2-\Delta\Delta}$Ct, where there is x = fold difference relative to the control.

Histological and immunohistochemistry analyses of kidney

The kidney tissue was dehydrated and embedded in paraffin, then sectioned at 5µm. The slides were subsequently stained with hematoxylin and eosin (H&E) and the morphological changes examined using a microscope (Eclipse 80i, Nikon, Japan), with images being captured by a digital camera (Fuij, Sapporo, Japan).

For immunohistochemistry, the paraffin-embedded renal sections were deparaffinized, rehydrated and immersed in H2O2 (3%) for ten minutes, in order to block any peroxidase activity. Following this, the slides were washed in phosphate buffer saline. Nonspecific binding sites were blocked by bovine serum albumin (5%) prior to the addition of TGF-1β polyclonal antibody in a dilution of 1:300 overnight at 4 °C. The slides were then washed in PBS and incubated with a secondary antibody, developed with 3.3'-diaminobezidine tetrahydrochloride and counterstained with hematoxyline.

Statistical analysis

Data are means ± standard error of six values collected from six different mice per each treatment. Data were analyzed using one-way ANOVA (analysis of variance) setting the probability level $P<0.05$, with the individual comparisons obtained by Duncan's multiple range tests for SPSS software version 11.5 for Windows (SPSS, IBM, Chicago, IL, USA). The probability level $P<0.05$ was considered statistically significant.

Results

The impact of Lesinurad and ALP on Liver and kidney biomarkers in hyperuricemic mice

HU demonstrated an increase in comparison to those of the control (CNT) group in the levels of liver biomarkers, uric acid and BUN. ALP and ZUR administered mice in the hyperuricemic group led to decreased GPT, GOT, uric acid and BUN [Figure 1A-B]. Co-administration of ALP and ZUR revealed an ameliorative synergistic effect ($P< 0.05$) on the normalization of GPT, GOT, uric acid and BUN levels (Figure 1A). It should be noted that AUR in HU mice revealed no higher level of influence than in ALP administered hyperuricemic rats.

The impact of Lesinurad and ALP on serum and hepatic XOD activity

As shown in Figure 2, there was an increase in the serum and liver XOD activities in hyperuricemic mice, which were significantly normalized in the ALP administered group as compared to ZUR administered hyperuricemic rats. Combination treatment, using both ALP and ZUR, induced an additive decrease in XOD activity compared to both hyperuricemic ALP and hyperuricemic ZUR treated groups.

The impact of Lesinurad and ALP on antioxidant activities altered by hyperuricemia

HU was found to increase tissue degradation, represented by an increase in levels of Malondialdehyde (MDA) (see Figure 3A), which was normalized in hyperuricemic mice by both ALP and ZUR treatment. It is of interest that HU decreased catalase and GPx levels were primarily readjusted following ALP administration, and to a lesser extent for ZUR administered groups (Figure 3B). Administration of ZUR to hyperuricemic mice, together with ALP, induced an additive ameliorative influence on the changes induced on MDA, catalase and GPx levels (Figure 3A, B).

The impact of Lesinurad and ALP on changes in cytokines altered in hyperuricemic mice

Figure 4 demonstrates the changes found in IL-1β and TNF-a protein levels. HU induced a state of inflammation, with a significant increase in levels of IL-1β and TNF-a ($P< 0.05$). It was found that the administration of ALP and ZUR to hyperuricemic mice normalized both IL-1β and TNF-a levels, while co-

administration of ALP and ZUR induced a greater (P<0.05) synergistic inhibitory effect on IL-1β and TNF-a (Figure 4).

The impact of Lesinurad and ALP on mRNA expression of renal genes associated with hyperuricemia

This current study examined the expression levels of renal mOAT-1, mOAT-3, mURTA-1 and mGLUT9, i.e. genes responsible for urate excretion and reabsorption in the kidneys. As shown in Fig. 5, in comparison to the mice in the control group, oxonate administration induced a significant down-regulation of mRNA expression of mOAT-1 and mOAT-3 in mice kidneys, alongside a significant up-regulation of the mURAT-1 and mGlut-9 expressions (p< 0.05). The alteration in the mRNA expression of urate transporter-related genes was consistent with the elevation of serum uric acid and BUN levels. ALP and ZUR treatment alone showed a significant down-regulation in mURAT-1 and mGlut-9 mRNA levels, as well as up-regulation in mOAT-1 and mOAT-3 expression (Figure 5). The additive synergistic influence on altered genes could be clearly observed when ALP and ZUR were co-administered to the hyperuricemic group.

The impact of Lesinurad and Allopurinol on renal histology and TGF-β1 immunoreactivity in hyperuricemic mice

Histopathological examination revealed that the kidneys of the control group demonstrated a normal histological picture, including normal glomerular and tubular architecture (Figure 6A). However, the kidneys of the hyperuricemic group revealed shrinkage of glomerular tufts with periglomrular and interstitial round cells infiltration. Tubular lumina showed obvious urate crystals occluding the lumina (Figure 6B). The kidneys of the ALP administered group demonstrated no marked change in renal histology (Figure 6C), while the kidneys of the ZUR administered group revealed degeneration of renal tubules with a few interstitial round cells infiltration (Figure 6D). The kidneys of the hyperuricemic group treated with ALP showed restoration of normal glomerular and tubular architecture (Figure 6E), while those administered only with ZUR demonstrated a slight restoration of a normal picture, with the presence of interstitial oedema (Figure 6F). The kidneys of the hyperuricemic group treated with both ZUR and ALP demonstrated a normal histological picture of both glomerular and tubular tissue, including an absence of urate crystals (Figure 6G).

Immunohistochemical kidney examination for TGF-β1 immunoreactivity revealed that the kidneys of the control group showed no immunoreactivity for TGF-β1 (Figure 7A). The kidneys of hyperuricemic group showed a clear and strong expression for TGF-β1 in kidney tissues (Figure 7B) and those of the ALP group showed no marked expression of TGF-β1 in renal tissue (Figure 7C). The kidneys of the ZUR group revealed a faint expression of TGF-β1 in renal tubular tissue, having moderate intensity (Figure 7D). In addition, the kidneys of hyperuricemic group treated with ALP demonstrated an absence of TGF-β expression in tubular tissue (Figure 7E). The kidneys of hyperuricemic group treated with ZUR alone

showed a prominent moderate intensity for TGF-β1 in kidney tissues (Figure 7F). However, the kidneys of the hyperuricemic group treated with ZUR and ALP showed glomerular and tubular tissue lacking in any TGF-β1 expression (Figure 7G).

Discussion

As noted above, hyperuricemia is a cause of gout, as well as a number of clinical disorders, including: (1) chronic kidney disease; (2) hypertension; (3) diabetes; (4) cardiovascular disorders; (5) dyslipidemia; (6) endothelial dysfunction; and (7) atherosclerosis. HU is associated with an increase in the production of oxygen free radicals, oxidative stress and up-regulation in pro-inflammatory cytokines and mediators [18, 19]. The management of gout, cardiovascular and metabolic disorders depends on the activity of xanthine oxidase. The key for hyperuricemia control includes the inhibition of an overproduction of uric acid, along with inflammation and oxidative stress [21].

As previously discussed, uric acid induces a state of inflammation in the kidneys and causes an inflammatory reaction. This accords with a previous study reporting relatively similar findings when employing Nuciferine [22]. A number of researchers have confirmed that gout shares many pathogenetic features associated with other inflammatory disorders, i.e. a rapid increase in the secretion of some pro-inflammatory cytokines (IL–1β, IL–6 and TNF-α) [23, 24]. The current study identified that the use of ALP or ZUR alone failed to reduce levels of IL–1β and TNF-α in hyperuricemic mice, suggesting that the co-administration of ALP and ZUR exerted their eliminate inflammation to prevent development of gout from HU. Moreover, an increase in levels of uric acid induced oxidative stress causing organ damage [25]. A significant decline was observed in the serum levels of catalase and GPx activities, accompanied by a significant increase in MDA levels in the hyperuricemic group in comparison to the control. ALP (either alone, or in combination with ZUR) significantly normalized, and reversed, changes in the measured serum levels of antioxidants and the lipid peroxidation marker in hyperuricemic mice, thus suggesting that ALP and ZUR increased antioxidant enzyme activities through their impact on oxidative stress biomarkers.

Xanthine Oxidase Inhibitors (i.e. ALP) are used as first-line therapy for patients with chronic gout, due to factors including availability, efficacy and low cost. However, ALP fails to lower the serum urate to the target level in a substantial subset of adherent patients. This results in the advice for Lesinurad therapy to be taken together with ALP. Lesinurad is a novel selective uricosuric, capable of overcoming the above limitations, while also proving effective in patients having an inadequate response to ALP monotherapy.

Efforts have been made, over a number of decades, to find a sufficient amount of urate lowering drugs. The close association between HU and metabolic and cardiovascular comorbidities has raised further interest in the development of novel urate-lowering drugs [26]. Uricosurics remain the second-line choice of treatment for HU and gout, with all recent prescriptions supporting the combination of uricosurics and ALP once monotherapy of each has proved ineffective [27].

Lesinurad is a selective URAT–1 inhibitor approved for HU treatment associated with gout, in combination with ALP. Its exact molecular mechanism is not fully elucidated in animal models. In clinical trials using healthy volunteers, a single dose of ZUR significantly reduced serum UA [27, 28], with its efficiency being due to approximately a third of the drug being excreted from the kidneys [28]. These current results prompted an investigation into the beneficial influence of ZUR on genes capable of validating the excretion and secretion of uric acid [29]. As noted previously, OAT1 and OAT3 are integrated in drug–drug interactions [29]. Lesinurad has been reported to regulate OAT–1 and OAT–3 expression *in vitro* studies [30]. The current study determined that Lesinurad has the potency to control OATs' expression to control HU *in vivo.*

Serum BUN levels form the markers of renal dysfunction. In addition, mURAT–1 is the main regulator for urate reabsorption (50%), playing a key role in the homeostasis of urate [31]. The glucose transporter 9 (GLUT9) is a protein responsible for urate reabsorption [32], while OAT–1 and OAT–3 are responsible for renal primary urate excretion [33]. This suggests that abnormalities in renal urate transporters may have important implications for the impairment of uric acid excretion, along with HU. The findings of the current study indicate that HU up-regulated mURAT–1 and mGLUT–9 and down-regulated mOAT–1 and mOAT–3, while co-administration of Lesinurad and ALP induced ameliorative synergistic effects. In addition, ZUR and ALP induced up-regulation in mOAT1 and mOAT–3 mRNA, alongside down-regulation in mURAT1 and mGLUT9 in the kidneys of hyperuricemic mice, thus indicating an enhancement of urate excretion reducing serum UA levels. These results confirm that ZUR demonstrates additional uricosuric effects in the presence of ALP, which (as confirmed by the current study) are mediated through renal mOAT1, mOAT–3, mURAT1 and mGLUT9 regulation in hyperuricemic mice.

HU results in fibrosis and injury renal tissues involving inflammation and fibroblast expansion. High levels of sodium in extracellular fluids are due to an increase in uric acid levels resulting in tissue nucleation [34], thus leading to inflammation influencing the biology of renal interstitial cells [34]. The increase in UA causes the expansion of fibroblasts, as well as up-regulation in the immunoreactivity of profibrotic factors (TGF-β1), confirming the activation of fibrotic pathways in hyperuricemic patients [35]. As demonstrated in Figure 7, this alteration in TGF–1β was confirmed during HU and normalization following co-administration of ZUR and ALP.

Conclusions

The present study confirmed the alterations induced in hyperuricemic mice, with the results showing ALP and ZUR co-administration being capable of lowering serum UA in hyperuricemic mice. In addition, they led to a decrease in the levels of XOD, oxidative stress, pro-inflammatory state, liver and kidney biomarkers. Furthermore, ALP and ZUR down-regulated the mRNA expression of URAT1 and GLUT9, while simultaneously up-regulating the mRNA expression of OAT1 and OAT–3 in hyperuricemic mice. The anti-hyperuricemic effect of ALP and ZUR is associated with the inhibition of XOD activity, resulting in a decrease in serum UA levels reported during HU. Furthermore, the co-administration of ALP and ZUR acted to improve kidney pathomorphology. All ZUR and ALP effects are summarized in Figure 8. This

study therefore suggests the advantages of the co-administration of ALP and ZUR for HU therapy. Furthermore, it provides evidence for the synergistic hypouricemic activity of both ALP and ZUR in the treatment of HU at the biochemical, molecular and cellular level in allopurinol-refractory hyperuricemic patients.

Declarations

Ethical Statement and Funding

The Scientific Deans of Taif University, Saudi Arabia, along with its Ethical Committee, approved all procedures and in vivo animal usage in this study for the project #1–439–6099, based on the NIH Guide for the Care and Use of Laboratory Animal. All precautions were followed to minimize animal suffering throughout the experiments.

Data Availability

Data are available

Author contributions

All authors contributed equally to this finished work: (1) YSA and MMS were responsible for the conception and design of the experiments; (2) MAN, YSA and MMS undertook the experiments; (3) MMS analyzed the data; (4) MMS undertook the biochemical assays; (5) MAN performed the histopathology; (6) MMS was responsible for the gene expression; and (7) YSA and MMS undertook the data interpretation.

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Conflict of Interest

The authors declare no conflict of interest.

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Table

Table.1. The primers used for quantitative real time PCR (qRT-PCR).

Gene	Product size (bp)	Accession number	Direction	Sequence (5'-3')
mOAT-1	183	NM_008766.3	Sense	GACAGGGTCTCATCCCTAGC
			Antisense	GTCCCTGACACACTGACTGA
mOAT-3	153	NM_001164635.1	Sense	TACAGTTGTCCGTGTCTGCT
			Antisense	CTTCCTCCTTCTTGCCGTTG
mURAT-1	145	NM_009203.3	Sense	GATAGGTTTGGGCGCAGAAG
			Antisense	TCATCATGACACCTGCCACT
mGlut-9	153	NM_001102415.1	Sense	TTCGGGTCCTTCCTTCCTCTA
			Antisense	GGACACAGTCACAGACCAGA
mb-actin	143	Nm_007393.5	Sense	CCAGCCTTCCTTCTTGGGTA
			Antisense	CAATGCCTGGGTACATGGTG

Figures

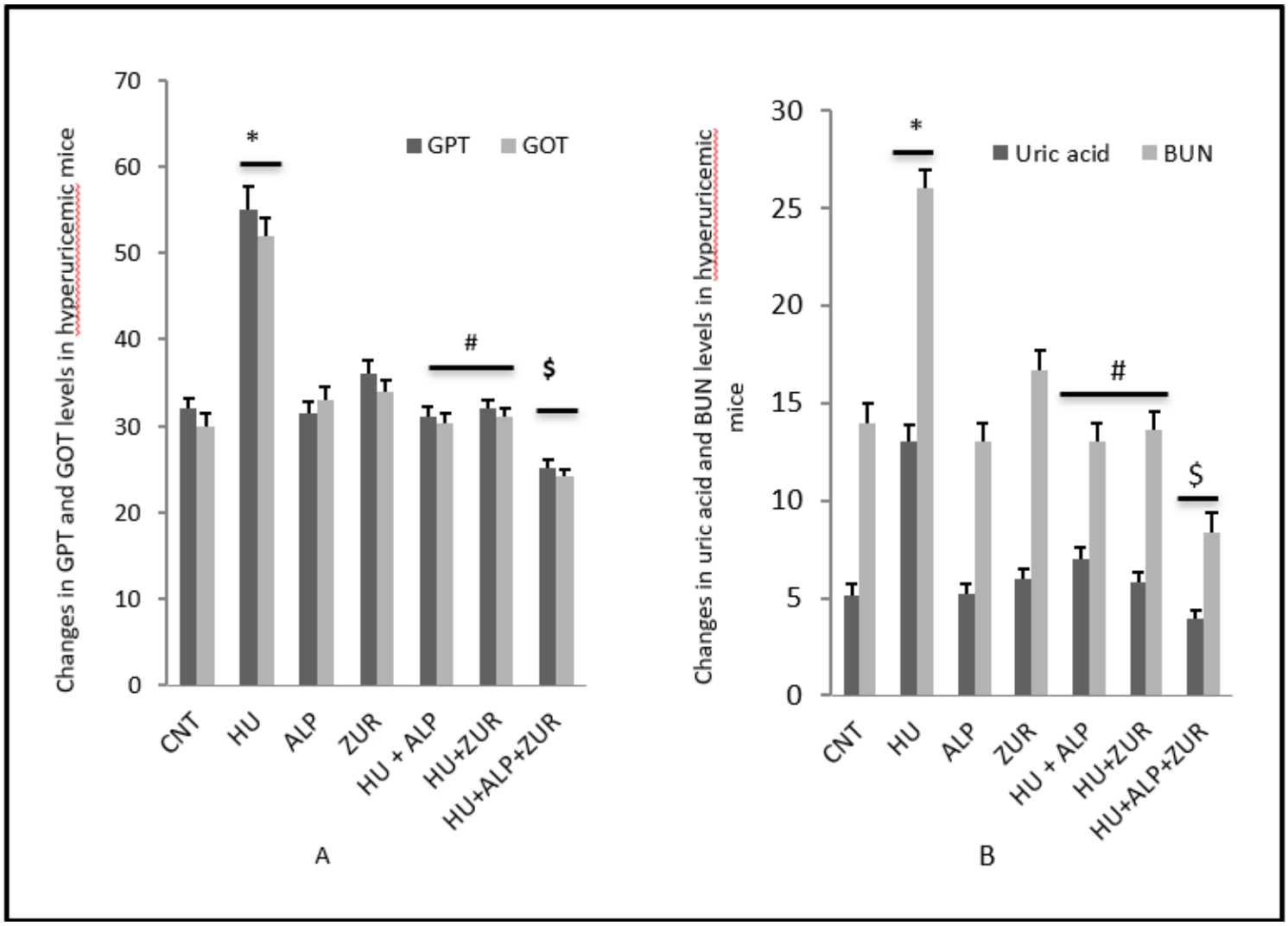

Figure 1

The impact of ZUR on changes in GPT, GOT, uric acid and BUN in hyperuricemic mice. Data are presented as means ± SE of six different mice. *p < .05 vs control group; #p < .05 vs HUR group and $p < .05 vis either HU+ ALP or HU + ZUR groups. CNT: control; HU: hyperuricemia; ALP ; ZUR. Units of GPT and GOT are U/L, and for uric acid and BUN are mg/dl.

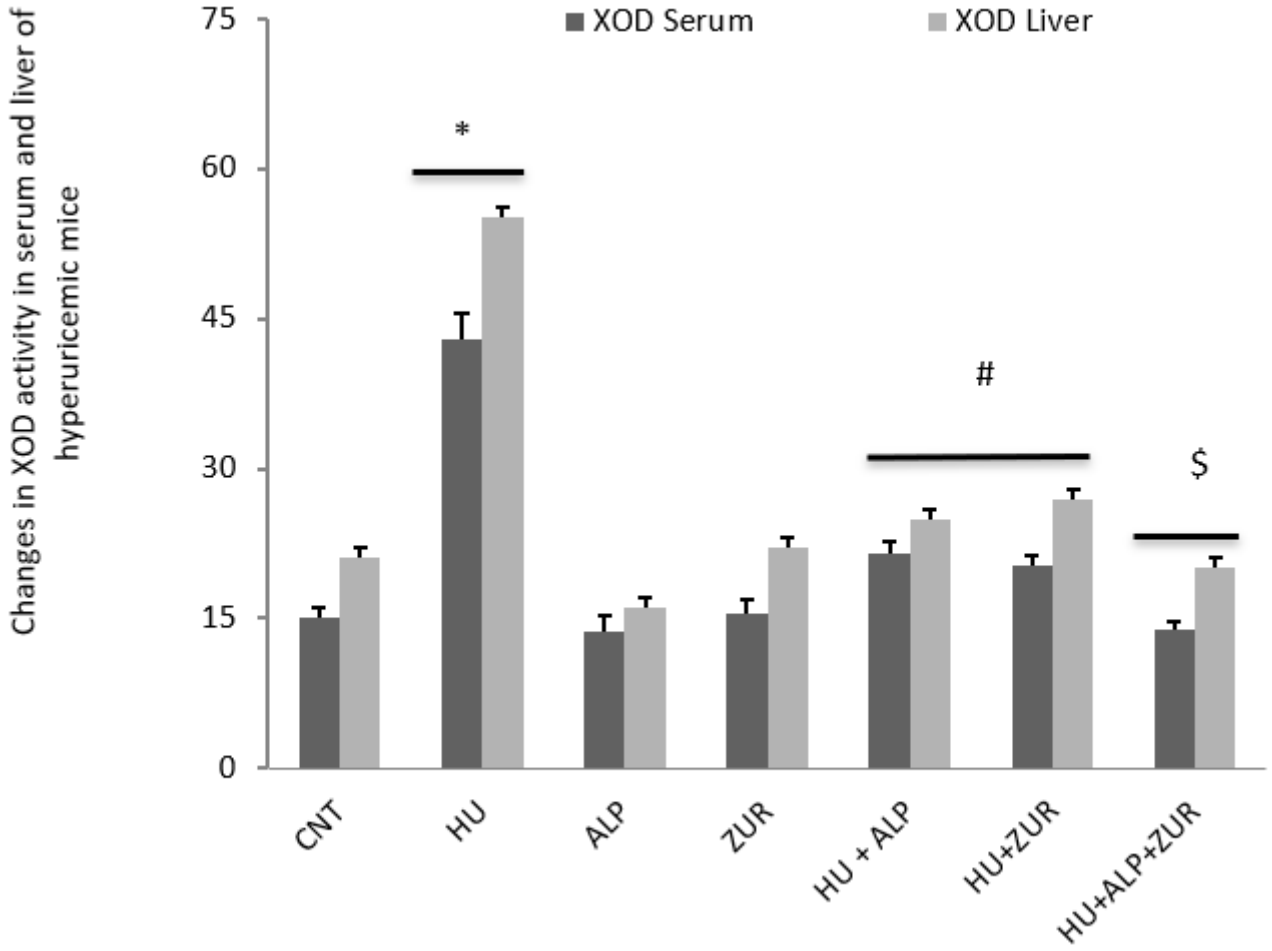

Figure 2

The impact of ZUR on changes in XOD activity in the serum and liver of hyperuricemic mice. Data are presented as means ± SE of five different mice. *p < .05 vs control group; #p < .05 vs HUR group and $p < .05 vis either HU+ ALP or HU + ZUR groups. XOD: xanthine oxidase; CNT: control; HU: hyperuricemia; ALP; ZUR. Measured unites for serum activity of XOD is U/l while for liver tissues is U/ g protein.

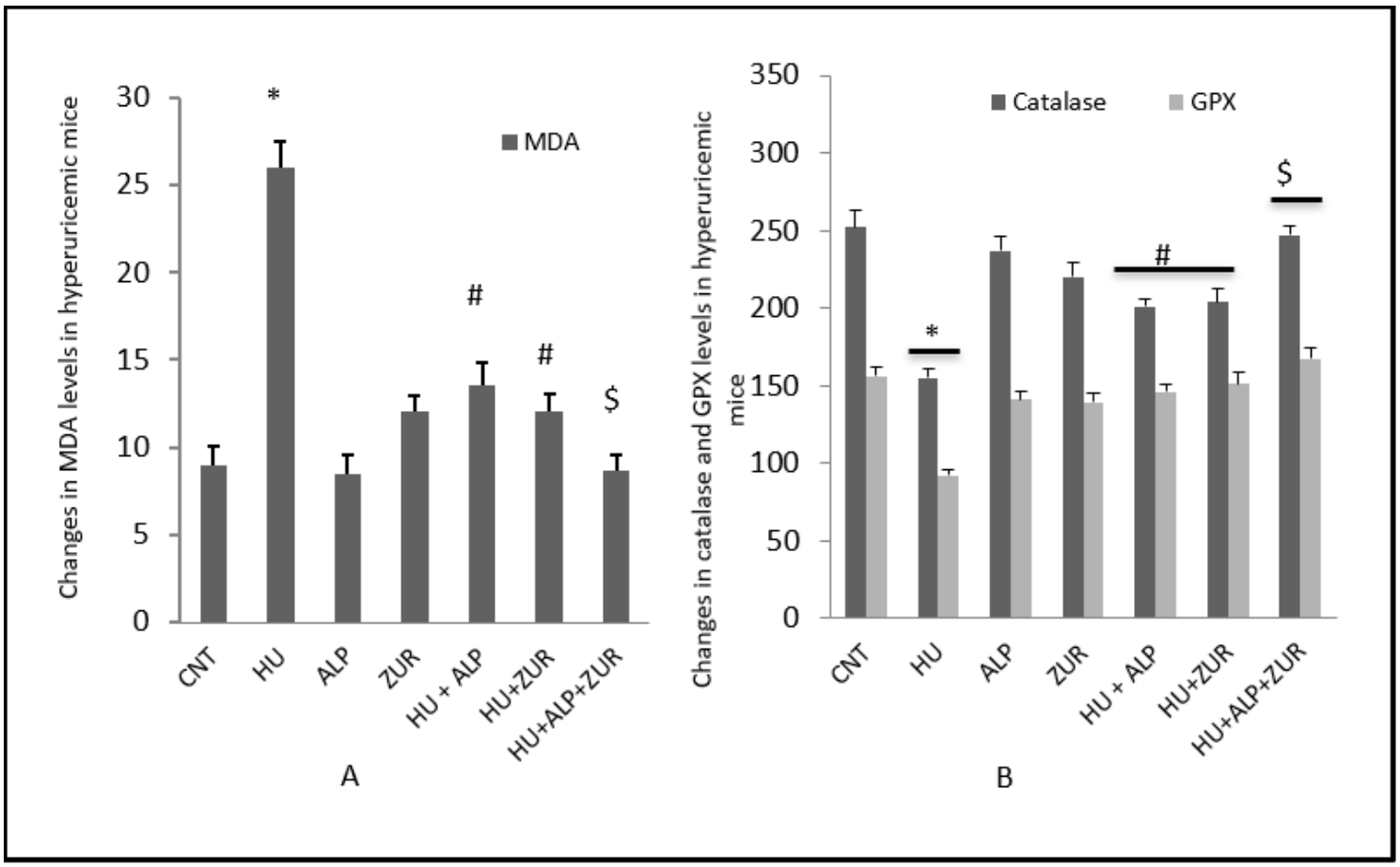

Figure 3

The impact of ZUR on changes in MDA, catalase and GPx in hyperuricemic mice. Data are presented as means ± SE of five different mice. *p < .05 vs control group; #p < .05 vs HUR group and $p < .05 vis either HU+ ALP or HU + ZUR groups. MDA: malondialdehyde; GPx: glutathione peroxidase; CNT: control; HU: hyperuricemia; ALP; ZUR. Units of MDA is nmol/ml, while for catalase and GPx are U/l.

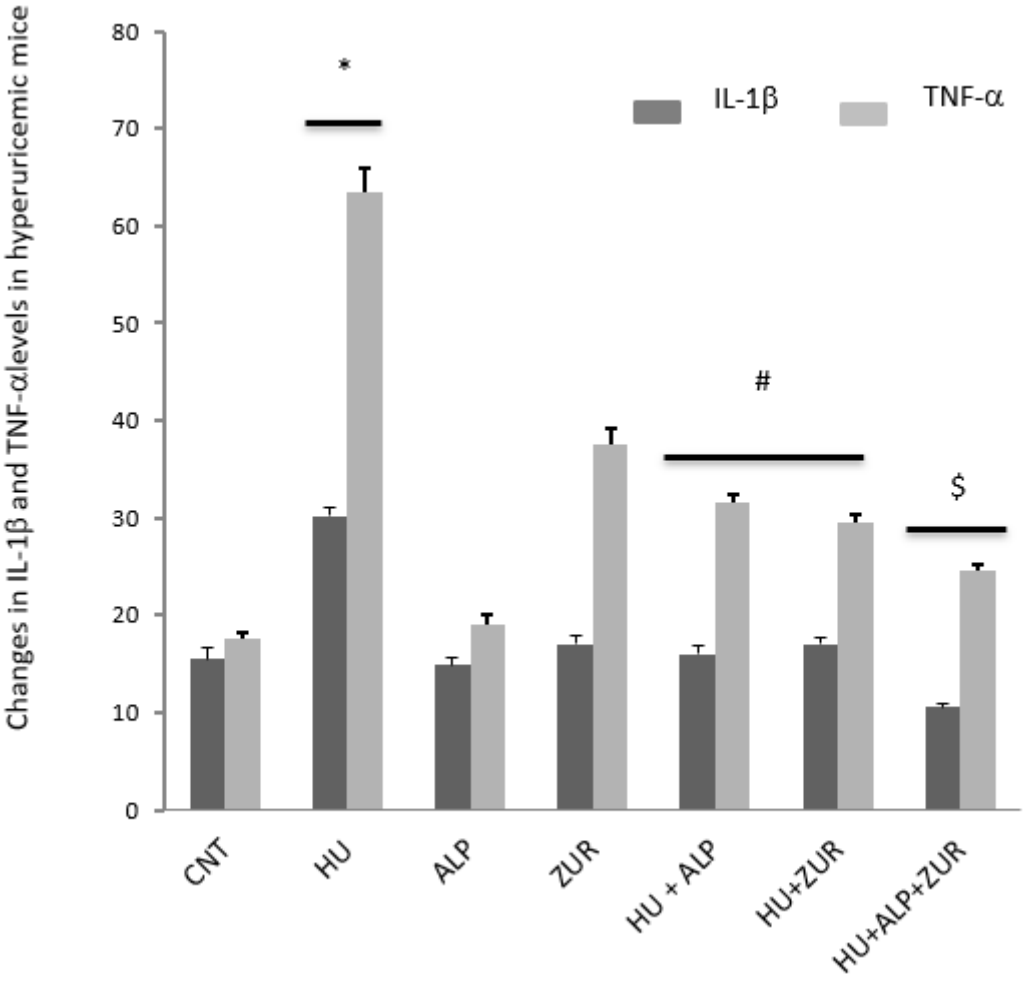

Figure 4

The impact of ZUR on changes in IL-1 and TNF- in hyperuricemic mice. Data are presented as means ± SE of five different mice. *p < .05 vs control group; #p < .05 vs HUR group and $p < .05 vis either HU+ ALP or HU + ZUR groups. IL-1: interleukin-1 beta; TNF-: tumor necrosis factor alpha; CNT: control; HU: hyperuricemia; ALP: l; ZUR. Measured units of IL-1 and TNF are pg/ml.

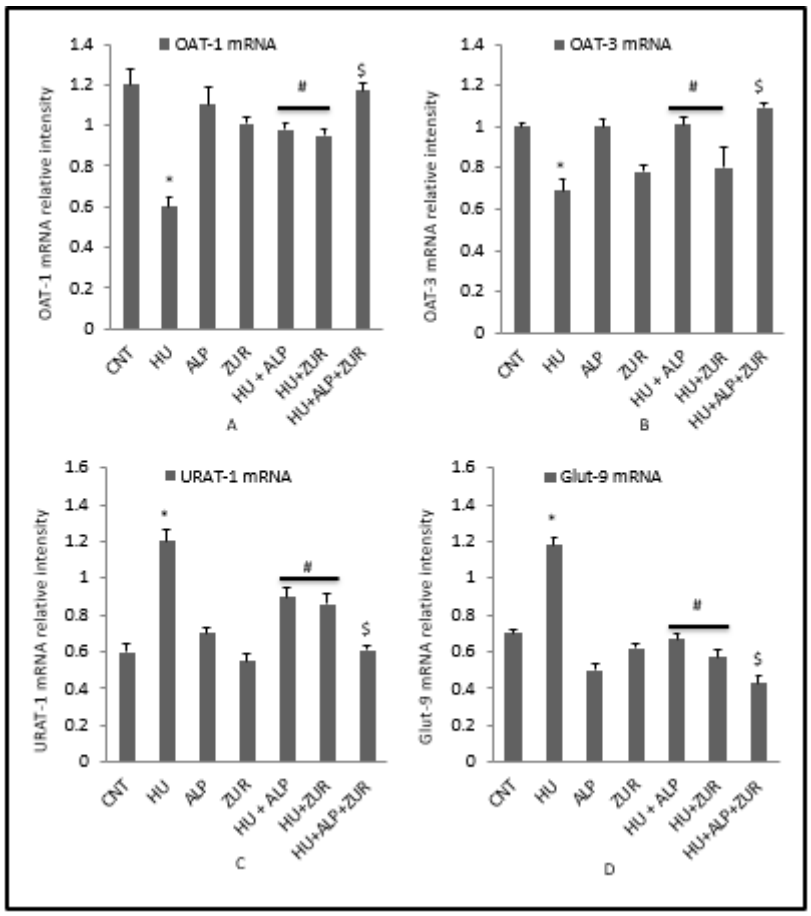

Figure 5

The ameliorative impact of ZUR on mRNA expression of OAT-1, OAT-3, URAT-1 and GLUT-9 in hyperuricemic mice by real time PCR. Graphic presentation of renal mRNA levels by real-time PCR analysis of OAT1 (A), OAT3 (B), URAT-1(C) and GLUT-9 (D) in different groups of mice after normalization with beta actin. *p < .05 vs control group; #p < .05 vs HUR group and $p <.05 vs either HU+ ALP or HU + ZUR groups.

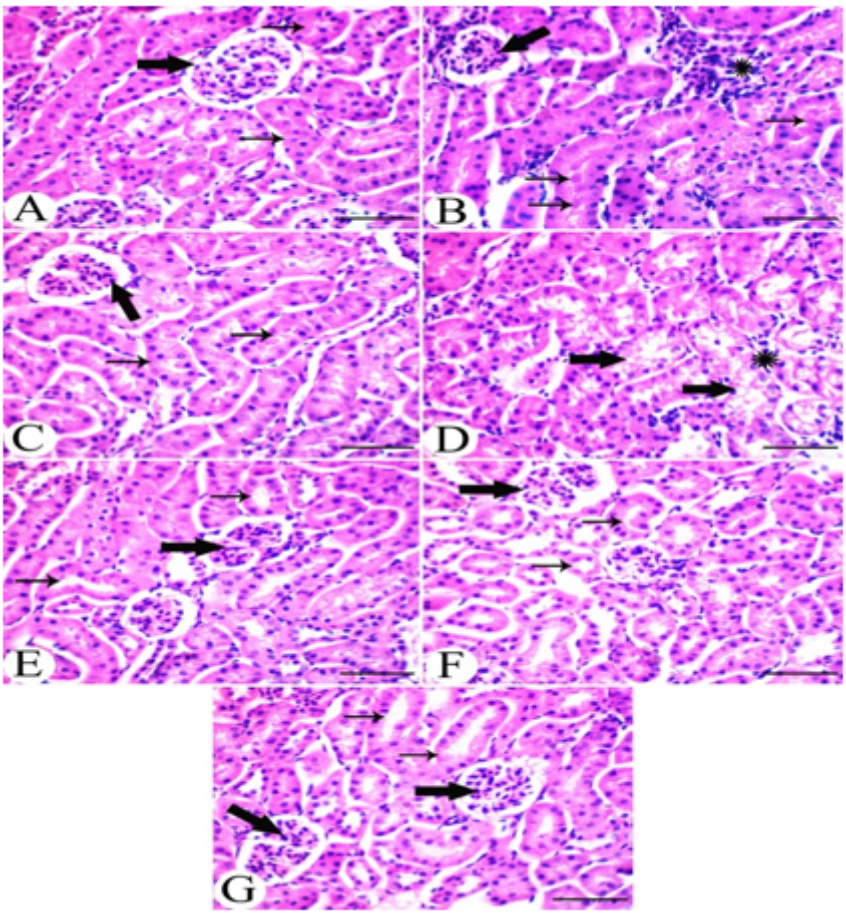

Figure 6

Histopathological examination of Kidneys. A. A kidney from the control group, showing a normal histological picture with normal glomerular (thick arrow) and tubular (thin arrows) architecture. B. A kidney from the hyperuricemic group, showing shrinkage of glomerular tufts (thick arrow) with periglomrular and interstitial (*) round cells infiltration. Tubular lumina showing obvious urate crystals occluding the lumina (thin arrows). C. A kidney from the ALP group, showing no marked change in renal histology with normal glomerular (thick arrow) and tubular (thin arrows) architecture. D. A kidney from the ZUR administered group, showing degeneration of renal tubules (thick arrows) with few interstitial round cells infiltration (*). E. A kidney from the hyperuricemic group, treated with ALP, showing restoration of normal glomerular (thick arrow) and tubular (thin arrows) architecture. F. A kidney from the hyperuricemic group, treated with ZUR alone, showing a slight restoration of normal glomerular (thick arrow) and tubular (thin arrows) picture with the presence of interstitial oedema. G. A kidney from the hyperuricemic group, treated with ZUR and ALP, showing a normal histological picture of both glomerular (thick arrow) and tubular (thick arrow) tissue, with the absence of urate crystals. H&E. Scale bar= 50 μm.

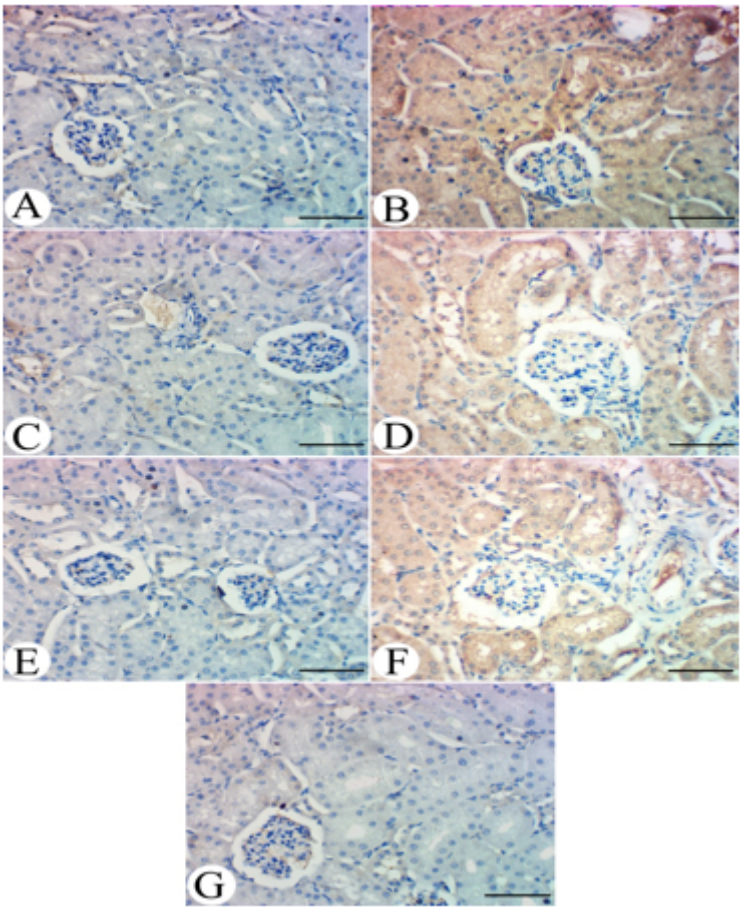

Figure 7

Immunohistochemical examination of TGF-β1. A. A kidney from the control group showing no expression of TGF-β in renal tissues. B. A kidney from the hyperuricemic group, showing a prominent expression of TGF-β1 in renal tubular tissue. C. A kidney from the ALP group, showing no marked expression of TGF-β1 in renal tissue. D. A kidney from the ZUR administered group, showing prominent expression of TGF-β1 in renal tubular tissue with moderate intensity. E. A kidney from the hyperuricemic group, treated with ALP shed absence of TGF-β1 immunoreactivity in tubular tissue. F. A kidney from the hyperuricemic group, treated with ZUR alone, showing a prominent moderate intensity of TGF-β1 in renal tissue. G. A kidney from the hyperuricemic group, treated with ZUR and ALP, showing glomerular and tubular tissue with no TGF-β1 expression. H&E. Scale bar= 50 μm.

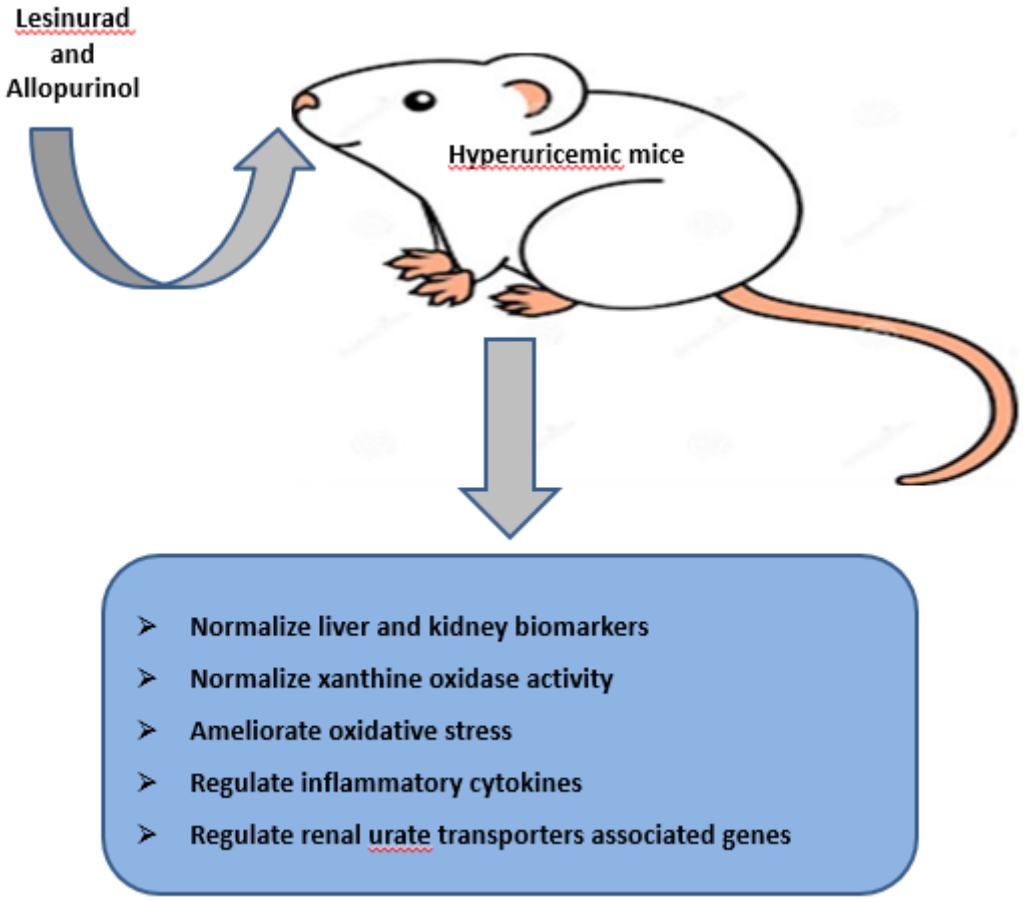

Figure 8

Schematic illustration for the ameliorative effects of lesinurad and allopurinol on hyperuricemia.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ArriveChecklist.pdf