International Journal of Biotechnology and Biomedical Sciences
p-ISSN 2454-4582, e-ISSN 2454-7808, Volume 3, Issue 1; January-June, 2017 pp. 71-77

http://www.krishisanskriti.org

Xanthine Oxidase: A Versatile Enzyme as a Future Therapeutic Target for Prevention of Inflammation Mediated Disorders

Safala Malvankar[1], Paramita Batabyal[2], Akshata Pahelkar[3] and Sadhana Sathaye[*4]

[1,2,3,4]Institute of Chemical Technology, Mumbai
E-mail: [1]safala.s.malvankar@gmail.com, [2]paramitabatabyal07@gmail.com
[3]akspahelkar@gmail.com, [4]sadhanasathaye@hotmail.com

Abstract—*Xanthine Oxidase (XO) also known as xanthine oxidoreductase (XOR) is a member of molybdoenzyme family and has a catalytic role in purine degradation, metabolizing hypoxanthine to xanthine and xanthine to uric acid (UA). It is a source of reactive oxygen species (ROS) and has several pathophysiological implications. XO is widely distributed in various organs especially liver and small intestine. It is also present in mammalian milk. There is detailed evidence about the enzyme's much broader biological role. ROS has implication in tissue, structural damage as well as cell signaling interference. XO plays an important role in the pathophysiology of disorders such as gout arthritis, diabetic complications, neurodegenerative and cardiovascular diseases. It will be interesting to study the substances interacting with this enzyme. Inhibition of this enzyme will inhibit ROS mediated disorders. XO, being a metabolizing enzyme, studies on the enzyme will indicate drug like properties of a substance as well as potential of a therapeutic candidate to produce drug interaction during polypharmacy. Deficiency of XO results in plasma accumulation and excess urinary excretion of xanthine, which may lead to arthropathy, myopathy, crystal nephropathy, urolithiasis, or renal failure. UA at physiological concentration possess antioxidant activity, however, higher levels demonstrate pro-oxidant effect, resulting in oxidative stress generation. Thus, homeostatic modulation of this enzyme will help healthy maintenance of physiological functions of the human body.*

1. INTRODUCTION

Xanthine Oxidase (XO) is a form of enzyme present in wide range of organisms from unicellular organisms (bacteria) to multicellular organisms. A century ago it was first identified in milk. In mammals, high levels of XO are found in liver and small intestine [2]. It is also present in rat liver, bovine heart, bovine capillary endothelial cells, human endothelial cells etc [3]. Previously, it has been stated that the enzyme XO is only present in cytosolic region [4] but recently it has also been reported to be present in the peroxisomes of hepatocytes and in various organelles of Kupffer and sinusoidal cells, including rough endoplasmic reticulum, lysosomes, and endocytic vesicles [5].

XO is a complex homodimer with catalytically independent subunits [6]. Each subunit has three domains associated with specific cofactors. The N-terminal domain is composed of two sub-domains, each with one iron sulphur (Fe_2-S_2) centre coordinated to four cysteine residues. The intermediate domain has a binding pocket for flavine adenine dinucleotide (FAD) that keeps the flavin ring in proximity to an (Fe_2-S_2) centre. The C-terminal domain is the largest domain and is associated with Mo–Co molybdopterin (Mo-Co) cofactor which is the active site of the enzyme [7].

XO is the metabolizing enzyme which is involved in terminal two reactions of purine catabolism pathway [2]. It catalyzes the oxidative conversion of hypoxanthine to xanthine and xanthine to uric acid (UA), the end product of purine catabolism in humans. The enzyme exists in two interconvertible forms Xanthine Dehydrogenase (XDH) and XO [8]. They differ primilarily in the oxidizing substrate specificity. During the conversion to UA, XO only reduces oxygen, whereas XDH can reduces either oxygen or NAD^+ but has greater affinity for NAD^+ [9]. In lower mammals UA further gets metabolized into allantoin by the enzyme urate oxidase but this enzyme is inactivated in humans [10]. Along with UA, the enzyme generates reactive oxygen species (ROS) such as superoxide radicals ($\cdot O_2^-$) and hydrogen peroxide (H_20_2). Excess UA and ROS production is responsible for release of lysosomal enzymes, which are the mediators of inflammation [11]. Inflammation is considered to be the major cause for various disorders such as diabetic complications, neurodegenerative disorders, gouty arthritis etc. Thus, certain measures are to be taken for treatment of these disorders by XO inhibitors. XO inhibitors are divided into two classes purine analogues and other is non- purine analogues. Examples of purine analogues are Allopurinol; Oxypurinol etc. which are the standard inhibitors of XO and non-purine analogues include Topiroxostat, Febuxostat etc. Also natural products based drugs have gained importance in reducing the

XO induced oxidative stress and inflammatory activity [11]. A good example of such drug is Quercetin [12].

2. PATHOPHYSIOLOGICAL IMPLICATIONS OF XO

2.1 Gout Arthritis

Gout Arthritis is a well known disease which is characterized by extreme pain in joints and kidneys due to the action of XO in generation of UA. Gout arthritis is associated with increase in the concentration of serum UA level leading to a condition known as hyperuricemia. Super saturation of body tissues with UA leads to deposition of monosodium urate crystals (MSU) in the joints of patients hence arising an inflammatory response [13]. The initial process of the gouty arthritis inflammatory response starts when macrophages that are present within the joint space phagocytose the MSU crystals. Phagocytosis of MSU crystals then triggers the formation of a protein scaffold known as an inflammasome within the cytosol of the macrophage. The inflammasome is a high-molecular weight protein complex that serves as a platform for the enzymatic processing of inactive pro-IL-1β into biologically active IL-1β, which is then secreted from the cell [14]. Interleukin (IL)-1β is crucial pro-inflammatory cytokine that regulates cell proliferation, differentiation, and apoptosis in gouty arthritis. The activated pro inflammatory cytokine IL-1β, binds to synovial endothelium and causes the release of IL-8 and tumor necrosis factor alpha (*TNF-α*).The release of pro-inflammatory cytokines is directly responsible for neutrophil influx to the synovium which is considered to be a hallmark of gouty arthritis. Recruitment of neutrophils causes further release of IL-1β and other pro-inflammatory cytokines that perpetuates gout arthritis and inflammation. MSU crystals alone may not be sufficient to trigger the activation or release of IL-1β from the macrophages, it requires co-stimulation with free fatty acids or lipopolysaccharide to release IL-1β [15]. It is reported that consumption of alcohol or a large meal can lead to increases in free fatty acid concentrations and hence can be a reason for involvement of free fatty acids in triggering release of IL-1β, an important factor in the development of gouty arthritis [15].

2.2 Diabetic Complications

Oxidative stress is considered to be an important factor in the progression of diabetic complications [16]. XO plays an important role in generation of free radicals in diabetic animals. XO is released by liver of diabetic animals [17]. Increased in superoxide radicals production in the vessel walls of aortic rings of diabetic rabbits explains the arterial complications of diabetes emphasizing the importance of XO in diabetes and its complications.

Oxidation of glutathione and lipoperoxidation is caused by oxidative stress in human type 1 diabetes. Inhibition of this enzyme prevents oxidative stress [17]. Allopurinol is found to be effective in prevention of this phenomenon. It was suggested that in type 1 diabetes, XO is released by the liver into plasma and then the enzyme binds with the glucosaminoglycans in the blood vessels and induce oxidative stress and tissue damage [18]. Heparin plays an important role in the regulation of lipid levels during diabetes. It was also found to decrease the peroxide levels in aortic rings from diabetic rabbits because it releases XO from the vessel wall. Hence heparin showed a new potential use in diabetes. Inhibition of xanthine oxidase has proved to be effective in improving endothelial vasodilator function in hypercholesterolemic, but not hypertensive patients [19]. Also it was reported that allopurinol protects against endothelial dysfunction in diabetic patients with mild hypertension [20].

Diabetic complications such as neuropathy, retinopathy, nephropathy etc. have significant correlation with increased level of uric acid [21]. The probable etiology factors of neuropathy involve Oxidative stress and inflammation. It is therefore speculated that UA generation by XDH/XO plays a role in diabetic neuropathy [21]. Focal UA production in the vitreous is thought to be involved in the pathogenesis and progression of Diabetic retinopathy [22]. A study states that elevated UA levels are a significant and independent risk factor for diabetic foot ulcer in female Chinese patients with type 2 diabetes mellitus [23].

2.3 Neurodegenerative Diseases

Oxidative stress plays a central role in a common pathophysiology of neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) [24]. AD is found to be affected approximately 16 million people worldwide. It is characterized by progressive loss of neurons associated with aggregation of protein as extracellular amyloid (βA) plaques, and intracellular tau tangles. The excessive accumulation of ROS in patients with AD may induce mitochondrial dysfunction. PD is the second most common neurodegenerative disease and is characterized by progressive loss of dopaminergic neurons in the substantia nigra, and aggregation of the protein α-synuclein. In PD brain, there is increased levels of 8-hydroxydeoxyguanosine [25], it has been reported that there is an increase in the common deletions in mitochondrial DNA in the surviving dopaminergic neurons in PD substantia nigra. Such deletions are believed to be the result of oxidative stress [26].

XO is one of the producers of ROS responsible for oxidative stress in mammalian brain. Under normal conditions the enzyme exists in the form of XDH. Hypoxia leads to metabolism of ATP and hence accumulation of hypoxanthine. When hypoxia persists, it induces a rise in intracellular calcium which activates the protease and converts XDH to XO in vivo and subsequent conversions of substrate into uric acid and ROS in brain [24]. Oxypurinol was found to be effective in reducing the production of ROS and protect neurons in the genetic presenilin 2 mouse model of AD [27] whereas allopurinol reduces *OH generation in rat striatum of toxic models of PD induced by paranonylphenol and MPP^+

[28], therefore indicating the role of XO in neurodegenerative diseases.

2.4 Cardiovascular Diseases

Oxidative stress contributes to endothelial dysfunction in cardiovascular diseases because superoxide radicals which are formed by the XO can readily inactivate endothelial nitric oxide (NO) thereby impairing vasorelaxation [29]. NO and $\cdot O_2^-$ react three fold greater than the rate at which superoxide dismutase (SOD) can eliminate $\cdot O_2^-$ resulting in endothelial dysfunction [30]. XO inhibition weakens the endothelial dysfunction. Allopurinol increases the NO stimulated blood flow in smokers [31] and diabetes [32] thereby reversing endothelial dysfunction. XO also contributes to endothelial dysfunction in hypercholesterolemia. Oxypurinol improved endothelial function in cholesterol fed rabbits [33, 34].

In coronary artery disease, XOR is co-localized in atherosclerotic plaques and due to this there is five to six fold elevated levels of UA in such plaques, indicating up regulation of XO activity [35].

XO is also involved in pathogenesis of hypertension. Tungsten rich diet, Oxypurinol and heparin binding SOD lowered the blood pressure in hypertensive rats [36]. It is reported that UA levels is correlated with blood pressure independent of XO activity [37].

3. XO: A METABOLIZING ENZYME

3.1 Drugs Metabolized By XO

3.1.1 Thiopurines: 6-Mercaptopurine (6-MP), Azathioprine (AZT) and 6-Thioguanine (6-TG)

6-MP, AZT and 6-TG, are the analogues of purine nucleosides that serve as the current childhood acute leukemia treatment. 6-MP, after administration, may enter into either anabolic or catabolic metabolic pathways. The anabolic pathway is responsible for conversion of 6-MP to its active form and the catabolic pathway converts the drug into inactive metabolites. This is achieved via two routes: One is thiol methylation, which is catalyzed by the enzyme thiopurine S-methyltransferase, to form the inactive metabolite methyl-6-mercaptopurine. The second inactivation route is the initial oxidation of 6- MP to 8-oxo-6-MP followed by conversion to 6-thiouric acid. 6-MP is converted to 6-thiouric acid via 6-thioxanthine which is catalyzed by XO in intestinal mucosa and liver. So with 6-MP, allopurinol is given in combination to inhibit XO catalyzed drug degradation [38, 39].

AZT is extensively converted to 6-MP which is then metabolized as mentioned above or it is directly oxidized to 8-oxo-azathioprine, catalyzed by Aldehyde oxidase (AO)-enzyme related to XO and then 8-oxo-6-mercaptopurine which may then be oxidized to 6-thiouric acid by XO [40]. AO is also involved in the metabolism of 6-TG [40].

3.1.2 Famciclovir

Famciclovir is a synthetic guanine derivative which is metabolized by XO or AO to the potent antiviral compound penciclovir. It is the active metabolite of famciclovir. It has its action against herpes simplex virus (HSV) types 1 and 2, varicella zoster virus (VZV), Epstein- Barr virus (EBV) and hepatitis B. Here, XO is considered important factor in converting the drug into its active metabolite and showing its particular action [41].

3.1.3 Acyclovir

Acyclovir is a guanosine analogue. It is a antiviral agent with high therapeutic index. It is effective in treatment of all types of HSV and VZV. The drawback of acyclovir is that, it does not have good water solubility. Therefore, it is given as a prodrug form, 6-Deoxyciclovir which is 18 times more soluble than acyclovir. The prodrug is extensively metabolized by XO in vivo and is converted to acyclovir. Here, XO is required as activating agent for the prodrug activation [42].

3.1.4 Carbovir

Carbovir is a carbocyclic guanosine derivative with a potent and selective inhibitory effect on HIV-1 replication. As its oral bioavailability is low, its prodrug, 6-deoxycarbovir was synthesized in attempt to increase its oral absorption [43].

6-deoxycarbovir is also reported as a XO activated prodrug but is a better substrate for AO as compared to XO [44].

3.1.5 Vidarabine

Vidarabine, a marine-derived drug has a antitumor activity. However, it is more noticeable for its antiviral activity with a high therapeutic index. Vidarabine possess significant activity against infections caused by herpes viruses, pox viruses, rhabdoviruses, hepadnaviruses, the vaccinia virus, VZV, and some RNA tumor viruses. Vidarabine is extensively deaminated in the body by adenosine deaminase (ADA) to 9-β-D-arabinofuranosyl hypoxanthine (hypoxanthine arabinoside) that has less antiviral activity than vidarabine, which is a major limitation in its clinical use [45]. Inhibition of ADA by co-administration of modified purine analogues with vidarabine, such as deoxycoformacine, can improve the therapeutic effect of vidarabine [46]. Vidarabine is rapidly converted by ADA to hypoxanthine arabinoside which has less antiviral activity than vidarabine. Hypoxanthine arabinoside is the major metabolite of vidarabine is excreted renally and biotransformed by XO to xanthine arabinoside. Allopurinol as an inhibitor of XO can interfere with the metabolism of vidarabine to produce toxicity and therefore, co-administration of allopurinol with vidarabine should be avoided or used with caution.

3.1.6 Theophylline

Theophylline is a methylxanthine used as a bronchodilator agent for the treatment of various asthmatic and pulmonary conditions. Theophylline is metabolized in the human liver through N-1 or N-3 demethylation followed by hydroxylation at C-8 producing three main products including 3-methylxanthine (3MX), 1-methyluric acid (1MU) and 1, 3-dimethyluric acid (1,3DMU). This reaction is catalyzed by that cytochrome P450 1A2 (CYP1A2) [47]. 1-Methyluric acid is the secondary metabolic pathway of theophylline. It is produced following a rapid oxidation from 1-methylxanthin by XO.1-methylxanthin is a good substrate for XO than other methylxanthines.Allopurinol as a potent inhibitor of XO activity decreases the formation of 1-methyleuric acid from the 1-methylxanthine in theophylline oxidation and produce toxicity [47].

4. DEFICIENCY OF XANTHINE OXIDASE

As discussed earlier, XO is considered to be a therapeutic target for the treatment of oxidative stress induced disorders. In contrast, to its involvement in many disorders, its deficiency in the body may lead to many disorders. Xanthinuria is an uncommon autosomal recessive disorder characterized by the excretion of urinary xanthine and hypoxanthine as the chief end products of purine metabolism and also the excess plasma accumulation resulting in low serum and urinary uric acid levels [48]. Deficiency of XDH or XOR may result into arthropathy, myopathy, crystal nephropathy, urolithiasis, or renal failure. Classic xanthinuria is one form of xanthinuria that is divided into 2 types based on the enzyme deficiency. Both types are inherited. Classic xanthinuria type I is caused by isolated deficiency of XDH and classic xanthinuria type II is caused by deficiency of XDH and AO. The other inherited form of xanthinuria, termed molybdenum cofactor deficiency. This defect is caused by mutations in molybdenum cofactor genes (MOCS1 and MOCS2) [48]. It occurs in the neonatal period with microcephaly, hyperreflexia, and other CNS manifestations. Other reported manifestations include severe metabolic acidosis and intracranial hemorrhage.

5. UA AS A PROTECTIVE AGENT???

Due to the anti oxidant mechanisms, our life span has increased remarkably. One such antioxidant mechanism is serum UA levels. UA is abundant antioxidant in plasma that scavenges singlet oxygen, peroxy radicals, and hydroxyl radicals. Uric acid can also bind with iron ion complexes, signifying additional antioxidant capabilities. Human plasma uric acid concentrations are typically high (≥ 2mg/dL) with a mean of 7.0mg/dL in men and 6.0mg/dL in women. UA has a concentration dependent effect in scavenging the radicals. It showed a decrease in DNA fragmentation and lipid peroxidation [49]. UA is as effective antioxidant as ascorbic acid and protect against oxidative damage [50]. It is reported that serum UA levels rise after an ischemic insult and elevated UA generates a protective response to oxidative stress [51]. In cerebral ischemia in rat models, there is increase in the levels of brain UA and in transient ischemia model, infusion of UA may lead to reduction of infarct volume and also improve behavioral outcome [52]. Further evidence suggested that intravenous infusion of UA after ischemic stroke reduce the markers of oxidative stress [53]. In addition, UA concentrations have been found to be reduced in Huntington's disease (HD) as compared to controls in several regions of the cerebral cortex. High UA levels slow the progression of PD in early PD [54]. Hence UA is beneficial in brain.

6. XO INHIBITORS

XO inhibitors are the drugs which lower the elevated uric acid levels in the body by inhibiting the XO. At an appropriate dose of XO inhibitors, uric acid levels return to normal. XO inhibitors are currently being investigated as potential therapeutics for disorders such as gout, hyperuricemia, ulcers, cancer and oxidative damage. XO inhibitors are of two classes: purine analogues and non purine analogues. Purine analogues include drugs such as Allopurinol, Oxypurinol, tisopurine and the second one is non purine analogues which include febuxostat, topiroxostat and inositols (phytic acid and myo-inositol). Non purine analogues have comparatively fewer side effects than purine analogue [55, 56].

Purine analogues	Non-purine analogues
Allopurinol–Standard Inhibitor	Febuxostat
Oxypurinol–Standard Inhibitor	Topiroxostat
Tisopurine	Inositols (Phytic acid and myo inositol)

Other non purine analogue drugs which are naturally occurring inhibitors are Quercetin (*Capparis spinosa*), Apigenin (*Matricaria recutita*), Thymol (*Thymus vulgaris*), Luteolin (*Lamiaceae* species), Cinnamaldehyde (*C. osmophloeum*), kaempferol (leaves of *Carica papaya*), ferulic acid (*Phaseolus vulgaris*) etc. Terpenoids like bisabolol are found to have a good XO inhibitory activity and has IC_{50} value of 34.70 µg/ml, as compared to allopurinol 8.48 µg/ml. Dietary polyphenolic compounds, mainly flavonoids, possess antioxidant properties and are potent inhibitors of xanthine oxidase (XO) activity. The combination of XO inhibitor with a compound which is capable of having a radical scavenger activity can be used for treatment of inflammatory conditions. This hypothesis is supported by the previously mentioned study where luteolin (as a inhibitor of XO) and epigallocatechin (as a superoxide scavenger) had been used as a combination therapy [57].

7. DISCUSSION

XO is homodimer protein with domain specific cofactors. It is found to have a broad substrate specificity. XO is widely localized in many tissues such as liver, small intestine,

mammary gland and abundantly identified in bovine milk. It is involved in terminal steps of purine degradation pathway converting hypoxanthine to xanthine and xanthine to UA .The enzyme generates ROS including superoxide radicals which along with UA is responsible for inflammation. XO is implicated in diseases such as gout arthritis, Diabetic complications, Neurodegenerative diseases and cardiovascular diseases. XO is an important metabolizing enzyme responsible for metabolism of various anticancer and antiviral drugs. XO plays an important role in generating an active or inactive metabolites of a particular drug or activation of a prodrug for its activity. AO: a XO related enzyme also is involved in metabolism of these drugs. XO inhibition is essential in inhibiting inflammation and ROS mediated disorders. Even though XO is implicated in above mentioned disorders, yet it is essential in the body. Its deficiency leads to a condition known as xanthinuria and related complications. Also UA is a protective agent through its antioxidant property reducing the oxidative stress in the body. Therefore a balanced level of XO should be maintained in the body in order to avoid the stated complications.

8. CONCLUSION

XO is a metabolizing enzyme involved in the last two steps of purine degradation pathway and generates ROS and UA, which in high concentration causes inflammation and this leads to many complications such as gout arthritis, diabetic complications, neurodegenerative disorders and cardiovascular diseases. Also, it plays an important role in the metabolism of many drugs. Therefore, XO inhibitors are essential to prevent inflammation and related disorders and also to prevent early metabolism of drugs. Considering the protective role of UA at lower physiological concentration, it is important to maintain homeostatic balance of XO in the body. XO can thus serve as a major target that can be explored in detail for drug discovery.

9. ACKNOWLEDGEMENTS

This work was supported in part by a grant from Department of Biotechnology (DBT). We thank SS laboratory colleagues, Ms Pritam Bagwe and Ms Anjana P Menon (M.tech Pharma Biotech, ICT Mumbai) for the valuable discussion.

10. REFERENCES

[1] F.ss Schardinger, “Ueber das Verhalten der Kuhmilchgegen Methylenblau und seine Verwendungzur Unterscheidung von ungekochterund gekochter Milch,” Zeitschrift für Untersuchung der Nahrungs- und Genussmittel, 1902 vol. 5, no. 22, pp. 1113–1121.

[2] Parks DA & Granger DN (1986). Xanthine oxidase: biochemistry, distribution, and physiology. *Acta Physiol Scand* **548**, 87–99.

[3] Jarasch ED, Bruder G & Heid HW (1986). Significance of xanthine oxidase in capillary endothelial cells. *Acta Physiol Scand* **548**, 39–46.

[4] Ichikawa M, Nishino T, Nishino T & Ichikawa A (1992). Sub cellular localization of xanthine oxidase in rat hepatocytes: high-resolution immunoelectron microscopic study combined with biochemical analysis. *J Histochem Cytochem* **40**, 1097–1103.

[5] Frederiks WM & Vreeling-Sindelarova H (2002). Ultrastructural localization of xanthine oxidoreductase activity in isolated rat liver cells. *Acta Histochem* **104**, 29–37.

[6] Krenitsky TA, Spector T & HallWW (1986). Xanthine oxidase from human liver: purification and characterization. *Arch Biochem Biophys* **247**, 108–119.

[7] Enroth C, Eger BT, Okamoto K, Nishino T, Nishino T & Pai EF (2000). Crystal structures of bovine milk xanthine dehydrogenase and xanthine oxidase: structure-based mechanism of conversion. *ProcNatl Acad Sci U SA* **97**, 10723–10728.

[8] Della Corte E & Stirpe F (1972). The regulation of rat liver xanthine oxidase: involvement of thiol groups in the conversion of the enzyme activity from dehydrogenase (type D) into oxidase (type O) and purification of the enzyme. *Biochem J* **126**, 739–745.

[9] Waud WR & Rajagopalan KV (1976*a*). Purification and properties of the NAD+-dependent (Type D) and O2 dependent (Type O) forms of rat liver xanthine dehydrogenase. *Arch Biochem Biophys* **172**, 354–364.

[10] Usuda N, Reddy MK, Hashimoto T, Rao MS & Reddy JK (1988). Tissue specificity and species differences in the distribution of urate oxidase in peroxisomes. *Laboratory Invest* **58**, 100–111.

[11] Shivraj Hariram Nile, Eun Young Ko, Doo Hwan Kim, Young-Soo Keum∗;Screening of ferulic acid related compounds as inhibitors of xanthine oxidase and cyclooxygenase-2 with anti-inflammatory activity; Brazilian journal of Pharmacognosy; 23 August 2015;VOL NO.26;Pg no-50-55.

[12] Bindoli A, Valenten M, Cavalini L; Inhibitory action of quercetin on xanthine oxidase and xanthine dehydrogenase activity; Pharmacol res.commun; 1985 Sep;17(9):831-9.

[13] Marcela C.P.M. Araújoa, Zilma S. Ferraz-Filhaa,b, Fernanda C. Ferrari a, Dênia A. Saúde-Guimarães; Campomanesia velutina leaves extracts exert hypouricemic effects through inhibition of xanthine oxidase and ameliorate inflammatory response triggered by MSU crystals;Brazilian journal of Pharmacognosy; 11 May 2016;Vol no.26 ;pg no 720-727.

[14] Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature. 2006; 440(7081):237–241.

[15] Joosten LA, Netea MG, Mylona E, et al. Engagement of fatty acids with Toll-like receptor 2 drives interleukin-1beta production via the ASC/caspase 1 pathway in monosodium urate monohydrate crystal-induced gouty arthritis. Arthritis Rheum. 2010; 62(11):3237–3248.

[16] Baynes J: Role of oxidative stress in development of complications in diabetes 1991. *Diabetes* 40:405–412.

[17] Marı´-Carmen Desco,1 Miguel Asensi,1 Rafael Ma´rquez,1 Jose´ Martı´nez-Valls,2 Ma´ximo Vento,1Federico V. Pallardo´ ,1 Juan Sastre,1 and Jose´ Vin˜a1; Xanthine Oxidase Is Involved in Free Radical Production in Type 1 Diabetes Protection by Allopurinol; DIABETES, VOL. 51, APRIL 2002.

[18] Adachi T, Fukushima T, Usami Y, Hirano K: Binding of human xanthine oxidase to sulphated glycosaminoglycans on the endothelial-cell surface; 1993; *Biochem J* 289:523–527.

[19] Cardillo C, Kilcoyne CM, Cannon RO 3rd, Quyyumi AA, Panza JA: Xanthine oxidase inhibition with oxypurinol improves

endothelial vasodilator function in hypercholesterolemic but not in hypertensive patients. *Hypertension* 30:57–63, 1997

[20] Butler R, Morris AD, Belch JJ, Hill A, Struthers AD: Allopurinol normalizes endothelial dysfunction in type 2 diabetics with mild hypertension. *Hypertension* 35:746–751, 2000.

[21] Akifumi Kushiyama, Kentaro Tanaka, Shigeko Hara, Shoji Kawazu; Linking uric acid metabolism to diabetic complications; World Journal of Diabetes; 2014 December 15; 5(6): 787-795.

[22] Krizova L, Kalousova M, Kubena A, Benakova H, Zima T,Kovarik Z, Kalvoda J, Kalvodova B. Increased uric acid and glucose concentrations in vitreous and serum of patients with diabetic macular oedema. *Ophthalmic Res* 2011; 46: 73-79.

[23] Ye X, Cao Y, Gao F, Yang Q, Zhang Q, Fu X, Li J, Xue Y. Elevated serum uric acid levels are independent risk factors for diabetic foot ulcer in female Chinese patients with type 2 diabetes. *J Diabetes* 2014; 6: 42-47.

[24] Sonia Gandhi, Andrey Abramov; Mechanism Of oxidative stress in neurodegeneration; Oxidative Medicine and Cellular Longevity Volume 2012 (2012), Article ID 428010, 11 pages.

[25] R. C. S. Seet, C. Y. J. Lee, E. C. H. Lim et al., "Oxidative damage in Parkinson disease: measurement using accurate biomarkers," Free Radical Biology and Medicine, vol. 48, no. 4, pp. 560–566, 2010.

[26] A. Bender, K. J. Krishnan, C. M. Morris et al., "High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease," Nature Genetics, vol. 38, no. 5, pp. 515–517, 2006.

[27] Y. Abe, Y. Hashimoto, Y. Tomita et al., "Cytotoxic mechanisms by M239V presenilin 2, a little-analyzed Alzheimer's disease-causative mutant," Journal of Neuroscience Research, vol. 77, no. 4, pp. 583–595, 2004.

[28] T. Obata, S. Kubota, and Y. Yamanaka, "Allopurinol suppresses para-nonylphenol and 1-methyl-4-phenylpyridinium ion (MPP+)-induced hydroxyl radical generation in rat striatum," Neuroscience Letters, vol. 306, no. 1-2, pp. 9–12, 2001.

[29] Cristine E. Berry and Joshua M. Hare; Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications; *J Physiol* 555.3 (2004), pp 589–606.

[30] Cai H & Harrison DG (2000). Endothelial dysfunction in cardiovascular disease: the role of oxidant stress. *Circ Res* 87,840–844.

[31] Guthikonda S, Sinkey C, Barenz T & HaynesWG (2003).Xanthine oxidase inhibition reverses endothelial dysfunction in heavy smokers. *Circulation* 107, 416–421

[32] Butler R, Morris AD, Belch JJF, Hill A & Struthers AD (2000).Allopurinol normalizes endothelial dysfunction in type 2 diabetics with mild hypertension. *Hypertension* **35**, 746–751.

[33] Ohara Y, Peterson TE & Harrison DG (1993). Hypercholesterolemia increases endothelial superoxide anion production. *J Clin Invest* 91, 2546–2551.

[34] White CR, Darley-Usmar V, Berrington WR, McAdams M,Gore JZ, Thompson JA, Parks DA, Tarpey M & Freeman BA(1996). Circulating plasma xanthine oxidase contributes to vascular dysfunction in hypercholesterolemic rabbits. *Proc Natl Acad Sci U S A* **93**, 8745–8749.

[35] Patetsios P, Song M, Shutze WP, Pappas C, Rodino W, Ramirez JA & Panetta TF (2001). Identification of uric acid andxanthine oxidase in atherosclerotic plaque. *Am J Cardiol* 88,188–191

[36] Nakazono K,Watanabe N, Matsuno K, Sasaki J, Sato T & Iwasaki T (2003). Does superoxide underlie the pathogenesis of hypertension? *Proc Natl Acad Sci U S A* 88, 10045–10048.

[37] Mazzali M, Hughes J, Kim Y, Jefferson A, Kang D, Gordon KL, LanHY, Kivlighn S & Johnson RJ (2001). Elevated uric acid increases blood pressure in the rat by a novel crystal independent mechanism. *Hypertension* 38, 1101–1106.

[38] Van Scoik, K.G.; Johnson, C.A.; & Porter, W.R. (1985). The pharmacology and metabolism of the thiopurine drugs 6-mercaptopurine and azathioprine. *Drug Metab Rev*, 16(1-2):157-174.

[39] Zimm, S.; Grygiel, J.J.; Strong, J.M.; Monks, T.J.; & Poplack, D.G. (1984). Identification of 6-mercaptopurine riboside in patients receiving 6-mercaptopurine as a prolonged intravenous infusion. *Biochem Pharmacol*, 33(24):4089-4092.

[40] Mohammad-Reza Rashidi and Roghiyeh Pashaei-Asl; Role of Aldehyde Oxidase and Xanthine Oxidase in the Metabolism of Purine-Related Drugs; World's largest Science, Technology & Medicine; published by intech.

[41] Rashidi, M.R.; Smith, J.A.; Clarke, S.E.; & Beedham, C. (1997). In vitro oxidation of famciclovir and 6 deoxypenciclovir by aldehyde oxidase from human, guinea pig, rabbit, and rat liver. *Drug Metab Dispos*, 25(7):805-813.

[42] Krenitsky, T.A.; Hall, W.W.; de Miranda, P.; Beauchamp, L.M.; Schaeffer, H.J.; & Whiteman, P.D. (1984). 6- Deoxyacyclovir: a xanthine oxidase-activated prodrug of acyclovir. *Proc Natl Acad Sci USA*, 81(10):3209-3213.

[43] Huang, S.H.; Remmel, R.P.; & Zimmerrman, C.L. (1991). The bioavailability and nonlinear clearance of (-)-carbovir in the rat. *Pharm Res*, 8:739-743.

[44] Vince, R.; Brownell, J.; & Beers, S.A. (1995). 6-Deoxycarbovir: a xanthine oxidase activated prodrug of carbovir, Nucleosides Nucleotides, 14, 39-44.

[45] Shen, W.; Kim, J.S.; Kish, P.E.; Zhang, J.; Mitchell, S.; Gentry, B.G.; Breitenbach, J.M.; Drach, J.C.; & Hilfinger, J. (2009). Design and synthesis of vidarabine prodrugs as antiviral agents. *Bioorg Med Chem Lett*, 19(3):792-796.

[46] Bryson, Y.; Connor, J.D.; Sweetman, L.; Carey, S.; Stuckey, M.A.; Buchanan, R. (1974). Determination of plaque inhibitory activity of adenine arabinoside (9-beta-Darabinofuranosyladenine) for herpes viruses using an adenosine deaminase inhibitor. *Antimicrob Agents Chemother*, 6(1): 98-101.

[47] Birkett, D.J.; Miners, J.O.; & Attwood, J. (1983). Secondary metabolism of theophylline biotransformation products in man--route of formation of 1-methyluric acid. Br J Clin Pharmacol, 15(1): 117-119.

[48] Rosa Torres Jiménez, Juan García Puig; Purine metabolism in pathogenesis of hyperuricemia and inborn errors of purine metabolism associated with the disease in Gout & Other Crystal Arthropathies, 2012.

[49] T Settle and H Klandorf*; The Role of Uric Acid as an Antioxidant in Selected Neurodegenerative Disease Pathogenesis: A Short Review; Settle and Klandorf, Brain Disord Ther 2014, 3:3.

[50] B. N. Ames, R. Cathcart, E. Schwiers, and P. Hochstein, "Uric-acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 78, no. 11, pp. 6858–6862, 1981.

[51] W. S. Waring, "Uric acid: an important antioxidant in acute ischaemic stroke," *QJM*, vol. 95, no. 10, pp. 691–693, 2002.

[52] Z. F. Yu, A. J. Bruce-Keller, Y. Goodman, and M. P. Mattson, "Uric acid protects neurons against excitotoxic and metabolic insults in cell culture, and against focal ischemic brain injury in vivo," *Journal of Neuroscience Research*, vol. 53, no. 5, pp.613–625, 1998.

[53] S. Amaro, D. Soy, V. Obach, A´. Cervera, A. M. Planas, and A´. Chamorro, "A pilot study of dual treatment with recombinant tissue plasminogen activator and uric acid in acute ischemic stroke," *Stroke*, vol. 38, no. 7, pp. 2173–2175, 2007.

[54] Peggy Auinger, MS1, Karl Kieburtz, MD1, and Michael P. McDermott, PhD1,2; The Relationship between Uric Acid Levels and Huntington's Disease Progression; Mov Disord. 2010 January 30; 25(2): 224–228.

[55] Koji Matsumoto, Ken Okamoto, Naoki Ashizawa, And Takeshi Nishino; Fyx- 051: A Novel And Potent Hybrid-Type Inhibitor Of Xanthine Oxidoreductase; The Journal Of Pharmacology And Experimental Therapeutics 2011; Jpet 336:95–103.

[56] Schumacher HR Jr. Febuxostat: A non-purine, selective inhibitor of xanthine oxidase for the management of hyperuricaemia in patients with gout. Expert OpinInvestig Drug. 2005; 14:893-903.

[57] Paul Cos, Li Ying, Mario Calomme, Jia P. Hu, Kanyanga Cimanga, Bart Van Poel, Luc Pieters,Arnold J. Vlietinck, and Dirk Vanden Berghe*; Structure-Activity Relationship and Classification of Flavonoids as Inhibitors of Xanthine Oxidase and Superoxide Scavengers; *J. Nat.*