Advances in Environmental Biology, 10(7) July 2016, Pages: 12-17

AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/

Evaluation of Serum Xanthine Oxidoreductases Enzymes in Cholelithiasis Patients: Correlation with Molybdenum and Iron

[1]Jwan Abdulmohsin Zainulabdeen and [2]Huda Ghazi Naser

[1]Department of chemistry, College of Science, University of Baghdad, Baghdad, Iraq.
[2]Department of chemistry, College of Science, Al-Nahrain University, Baghdad, Iraq.

Address For Correspondence:
Jwan Abdulmohsin Zainulabdeen, Department of chemistry, College of Science, University of Baghdad, Baghdad, Iraq.
E-mail: jwanbio2016@gmail.com.

Received 22 May 2016; Accepted 18 July 2016; Available online 8 August 2016

ABSTRACT

Background Cholelithiasis is one of the most prevalent gastrointestinal tract diseases, autopsy reports have shown a prevalence of cholelithiasis from 11% to 36 %, the female is three times more likely to develop this disease than male. The aim of this study is to verify possible relations between xanthine oxidoreductases enzymes, uric acid and trace elements (Mo and Fe) in sera of cholelithiasis patients and to evaluate the possibility of a new biomarker for the detection of this disease. **Materials and Methods** : in this study (76) of cholelithiasis patients, with (57) apparently healthy as control were enrolled in this study .Total serum protein, xanthine oxidoreductases enzymes [xanthine oxidase (XO) and xanthine dehydrogenase (XDH)], uric acid and trace elements (Mo and Fe) were measured. **Results:** high significant increases (p<0.01)) in XO activity, its specific activity , (XO/XDH) ratio, uric acid and trace elements for patients group in comparison with control were detected, while high significant decreases (p<0.01) in XDH activity, its specific activity and total serum protein in patients group were appeared in this comparison .**Conclusion**: The results of study were indicated that, highest XO/XDH ratio in patients confirm the idea of increase conversion rate of XDH to XO in this pathogenic condition**,** meanwhile free radical production increased so oxidative stress increase, and catabolic pathway of hypoxanthine and xanthine was increased, this increase may confirmed by increasing in uric acid levels. Meanwhile XO activity may be used as diagnostic tool for this disease.

KEYWORDS: Cholelithiasis, Xanthine oxidoreductases, Molybdenum**,** and Iron.

INTRODUCTION

The presence of stones in the gallbladder is referred to as cholelithiasis or gallstone disease (GD) [1]; it is one of the most prevalent gastrointestinal tract diseases, with a substantial burden to healthcare systems [2]. Autopsy reports have shown a prevalence of cholelithiasis from 11% to 36 %, the first-degree relatives of patients with cholelithiasis have a twofold greater prevalence. Gallstone (GS) can vary in shape and size from as small as a grain of sand to as large as a golf ball, also they classified by their cholesterol content as either cholesterol stones or pigment stones [3, 4] In approximately 70% of cases, nonspecific GS-associated symptoms occur [5,6], the symptom most closely associated with cholelithiasis disease is (1–24) hour lasting abdominal pain with radiation to the upper back. Onset more than an hour after meals support this diagnosis, meanwhile, symptoms of cholelithiasis may include: pain in the abdomen and back is infrequent but severe, the increase in abdominal pain after eating a fatty meal, fever and pain, if the GB or bile duct becomes infected and jaundice[7,8].

Xanthine oxidoreductase (XOR) is part of a group of enzymes known as the molybdenum iron-sulfur flavin hydroxylases; it is an evolutionarily conserved housekeeping enzyme, with a principal role in purine catabolism by catalyzing the two last steps in purine catabolism, forming uric acid from hypoxanthine and xanthine. XOR exists in two distinct functional but interconver-tible forms: xanthine oxidase (XO; xanthine- oxygen oxidoreductase; EC (1.17.3.2), which catalyzes reactions 1 and 2, and xanthine dehydrogenase (XDH; xanthine-NAD oxidoreductase; EC (1.17.1. 4), which catalyzes reaction 3.

$$\text{Xanthine} + 2\ O_2 + H_2O \leftrightarrow \text{uric acid} + 2\ O_2^{-} + 2\ H^{+} \quad (1)$$
$$\text{Xanthine} + O_2 + H_2O \leftrightarrow \text{uric acid} + H_2O_2 \quad (2)$$
$$\text{Xanthine} + NAD^{+} + H_2O \leftrightarrow \text{uric acid} + NADH + H^{+} \quad (3)$$

Although the XDH is the most abundant form *in vivo*, it's easily converted to XO by oxidation of the sulfhydryl residues (reversible) or by proteolysis (irreversible). XO contains one Molybdenum, one of the flavin adenine dinucleotides (FAD), and two iron-sulfur (2Fe-2S) centers of the ferredoxin-type in each of its two independent subunits. The enzyme contains two separated substrate-binding sites. XO and XDH mainly differ in the structure of the flavin adenine dinucleotide (FAD) domain which affects their choice of electron acceptors. XOR up-regulated by either inflammatory mediators or hypoxia and, in the presence of molecular oxygen, reduces the oxygen to reactive oxygen species (ROS) [9]. Different forms of stimuli induce the conversion of the XD to the XO form, presumably resulting in an intensive synthesis of ROS and reactive nitrogen species (RNS) [10]. As a result of a selective proteolysis, NAD^{+}-reducing XDH converted to oxygen-reducing XOD, when tissue reperfused (molecular oxygen reintroduced into the tissues), oxygen interacts with hypoxanthine and XOD to produce ($O_2^{\cdot -}$). Once formed, superoxide converts to H_20_2 and the hydroxyl radical; together, these compounds cause oxidant tissue injury. According to the sequence of events during the ischemic period, ATP is catabolized thereby depleting ATP to hypoxanthine, which accumulates in the tissues. A consequence of this energy depleted state is the increased influx of Ca^{+2} into the cell. The increased intracellular Ca^{+2} then trigger the conversion of XDH to XOD [11].

Trace elements have been extensively studied in recent years to assess whether they have any modifying effects in the etiology of the disease. It has become well established that many trace elements play an essential role in some biological processes; they usually associated with the protein (metalloprotein) or an enzyme (metalloenzyme) as a principal components or cofactor. The action of a slight amount of trace elements is necessary for optimal performance of the whole organism. The basis for the amplification of trace element action is related to their interaction with enzymes and hormones, which regulate the metabolism of much larger amounts of biochemical substrates [12].

This study aimed to assess relations between xanthine serum xanthine oxidoreductases, uric acid and trace elements (Mo and Fe) in sera of cholelithiasis patients.

MATERIALS AND METHODS

A total of Seventy six of cholelithiasis patients and apparently fifty seven healthy as control were comprised in this study, the age of these patients ranged from (41 year to 50) years . Five to Ten millilitres of blood were collected into test tubes without anticoagulants, after coagulation, the blood samples were centrifuged. The sera were stored at (-20°C), to be used for the studied parameters estimation. All patients were subjected to a personal interview using especially designed questionnaire format full history with detailed information.

Determination of Some Biochemical Parameters:

Xanthine oxidase activity (XO) was determined by the method of Ackermann and Brill [13]. Dehydrogenase (XDH) activity of xanthine was determined by Fried *et al*. method [14]. Flameless atomic absorption spectrophotometer is the recommended technique for (Mo, Fe). Uric Acid was measured by a spectrophotometric method [15].Total serum protein (T.S.P.) concentration was determined by Modified Biuret method [16].

Statistical Analysis:

Data were processed with the software package SPSS Ver.21 [SPSS Inc. Chicago IL] and Microsoft Excel XP version. Descriptive analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was estimated by Student T-Test. The probability ($p< 0.05$) = significant, ($p<0.01$) = high significant, ($p> 0.05$) = non-significant. ANOVA test was used to show the differences between variables of differentiated groups. Correlation analysis was used to test the linear relationship between parameters.

RESULTS AND DISCUSSION

In the present study, (133) individual samples were included, the control group consist of (57) apparently healthy individual samples, while the cholelithiasis patients were (76) individual samples. As shown in Table (1), the mean value of XO activity, specific activity of XO (S.A. XO), (XO/XDH) ratio, and also mean levels of uric acid were revealed high significant increases ($p<0.01$) for patients group when compared to control group, in contrast T. S.P., XDH activity and specific activity of XDH (S.A XDH) were found to be high significant decreases ($p<0.01$) for patients group in comparison with control.

Table 1: Total Serum Protein, Activities and Specific Activities of Serum Xanthine Oxidase, Xanthine Dehydrogenase, and Uric Acid for all Control and Patients groups.

Parameters	Control (n=57) Mean ± SD	Patient (n=76) Mean ± SD	Comparison of Sig.	
			p value	Sig
T.S. P (g/dL)	7.335 ± 0.376	7.015 ± 0.396	$p<0.01$	S
XO activity (U/L)	21.921 ± 7.548	80.020 ± 31.564	$p<0.01$	S
S.A. XO (U/g)	0.299 ± 0.104	1.141 ± 0.451		
XDH activity (U/L)	2.436 ± 1.636	1.529 ± 0.619	$p<0.01$	S
S.A. XDH (U/g)	0.033 ± 0.023	0.022 ± 0.009		
XO/XDH ratio	11.565 ± 7.661	66.937 ± 5.281		
Uric acid (mg/dL)	4.436 ± 1.142	5.067 ± 1.251	$p<0.01$	S

The results of mean trace elements (Fe, Mo) concentrations in sera of patients showed a high significant increases ($p<0.01$) in both (Fe, Mo) when compared to control group as shown in table (2).

Table 2: The Mean Values of Trace Elements for all Control and Patients Groups.

Parameters	Control (n=57) Mean ± SD	Patient (n=76) Mean ± SD	Comparison of Sig.	
			p value	Sig
Fe (µg/ml)	2.520 ± 0.796	6.710 ± 6.710	$p<0.01$	S
Mo (µg/ml)	0.012 ± 0.004	0.029 ± 0.010	$p<0.01$	S

Cholelithiasis cause no symptoms, even for years; therefore, called "silent stones" and do not require treatment, however to our knowable this is the first attempt to measure this activity XO and XD activities in cholelithiasis patients. In present study, highly significant increases ($p<0.01$) were showed in activities and specific activities of XO, in contrast a highly significant decreases ($p<0.01$) in the activities and specific activities of XDH were found in sera of cholelithiasis patients group in comparison to control group. Also the results of our study show the highest XO/XD ratio in cholelithiasis patients which confirm the idea of increase the rate of conversion of XD to XO in this pathogenic condition in parallel the free radical production increased, and so the oxidative stress increase .Several mechanisms have been proposed to be involved in the generation of oxygen free radicals but XO has been shown to be a major source of free radical generation under ischemic conditions. Our results are in agreement with many other studies [17] which suggested that the overall purine enzymatic pattern confers selective advantages to disease cells by making them more efficient for retention and production of precursors for synthesis of purine and pyrimidine nucleotides and subsequently, for RNA and DNA biosynthesis, it was suggested that oxidative stress might be increased in abnormal conditions and may affect the course of the disease. On the other hand when the oxidative stress is higher, alteration in some purine metabolizing enzymes was found. The high XO activity may be an attempt to lower salvage pathway activity for purines, which is vital for rapid DNA synthesis. Congenital diseases may also give rise to hyperuricemia, recessive disorders involving the overproduction of uric acid due to complete or partial lack of hypoxanthine phosphoribosyl-transferase (HPRT) [18], which acts to salvage purines from degraded DNA, taking intracellular hypoxanthine to inosine monophosphate (IMP) and xanthine to xanthine Monophosphate (certain isozymes), and a deficiency or absence of this enzyme results in elevated concentrations of XOR substrates in the cell [19, 20]. Treatment with an XOR inhibitor largely prevents the development of Diseases [21-23].

Uric acid a breakdown product in ingested and endogenously synthesised purines, DNA and RNA are degraded into purine nucleotides and bases, which are then metabolised, via the action of xanthine oxidase, to xanthine and then uric acid [24]. In humans uric acid undergoes no further metabolism. and homeostasis relies upon excretion uric acid, predominantly via the kidneys, and humans and have the ability to reabsorb uric acid in the proximal tubule, via the action urate transporter as the major roles of xanthine oxidase. is to conversion of hypoxanthine to xanthine and to uric acid, an interconvertible form, xanthine dehydrogenase, also exists and is responsible for conversion of NAD^+ to NADH.The action of these enzymes yields hydroxyl free radicals and hydrogen peroxide which can add to or initiate oxidative stress. In this study, an increase in sera uric acid concentrations were observed in cholelithiasis patients, this increase may be due to the parallel increasing in xanthine oxidase activity observed in gallstone patients reflect the fact that the catabolic pathway is increased. In this study the results show a decrease in the concentration of serum total protein in patients when compared to that of control. In general terms, variations in plasma protein concentrations can be due to any of three changes.

in the rate of protein synthesis, the rate of their removal, and in the volume of distribution .So, the differences in total serum proteins pattern may be explained mainly by the differences in serum albumin concentrations, and synthesis of albumin was reported to be reduced in case of hereditary defects, liver diseases, malnutrition and another disease[25].

Like iron, copper, zinc, manganese, and cobalt, molybdenum (Mo) can be utilized as a stably bound, variably coordinated cofactor in proteins, in mammals, Mo is found in three different enzymes: aldehyde oxidoreductase (AOR), sulfite oxidase (SOX), and xanthine oxidoreductase (XOR). Humans possess each of these enzymes, which differ slightly in the coordination of the Mo -containing cofactor Molybdenum by itself is relatively inert in biological processes, and requires an additional cofactor to be biologically active in molybdo-enzymes [26]. The results of our study indicated that high significant increase ($p<0.01$) in Mo levels in patients group when compared to control group that may be because increasing the activity of XO which is directly proportional to the amount of Mo in the body. However, an extremely high concentration of Mo reverses the trend and can act as an inhibitor in both purine catabolism and other processes. Mo concentrations also affect protein synthesis, metabolism and growth [27], and that may also prove the increase the rate of conversion of XDH to XO in this disease.

Iron is abundant in biology, Iron-proteins are found in all living organisms, ranging from the evolutionarily primitive archaea to humans [28]. Iron-containing enzymes and proteins, often containing heme prosthetic groups, participate in many biological oxidations and in transport. Examples of proteins found in higher organisms include hemoglobin, cytochrome P450 and catalase .Most of the iron (Fe^{2+}) is oxidized to (Fe^{3+}) by the ferroxidase activity of ceruloplasmin (Cp) and /or spontaneous oxidization and then bind to transferrin and to be acquired by the cells. However under pathological conditions the loss of Cp ferroxidase activity make it impossible for most ferrous ion to be oxidized to ferric ion: accordingly, the amount of ferric ion and transferrin – bound Fe^{3+} will decrease, while non- transferrin – bound iron such as citrate –Fe^{2+}, ascorbate –Fe^{2+} and free ferrous iron will increase, this will induce oxidative stress and free radical formation, and trigger a cascade of pathological events leading to cell death. It is also possible that the rate of spontaneous oxidization of ferrous ion to ferric ion will increase so that more (Fe^{3+}) can be formed, as well as, generate a large amount of (ROS) [29]. The results of our study indicated that high significant increase ($p<0.01$) in Fe levels in patients group when compared to control group that may be because increasing the activity of XO as it is as well as Mo components of enzymes.

According to our results there were some significant correlations as follows: (A) positive correlation between XO with S.A. XO, (B) negative correlation between S.A. XO with BUN, (C) positive correlation between XDH with S.A. XDH, (D) negative correlation between S.A. XDH with XO/XDH ratio, (E) positive correlation between XO/XDH ratio with uric Acid and, (F) positive correlation between XO/XDH ratio with Mo, as shown in figure (1).

Serum XO activity were showed a significant positive correlation with S.A XO figure (1,A), this supports our results in Table (1) if the XO activity increased its S.A. increased too, and serum S.A XO showed negative correlation with urea figure (1,B), urea is made when protein is broken down in body and is made in the liver and passed out of body in the urine if there is any problem in broken down for protein the level of urea will be change that is may be the cause for negative correlation with urea, serum XDH showed a significant positive correlation with S.A XDH figure (1,C) this supports our results in Table (1) if the XDH activity increased its S.A. increased too, serum S.A XDH showed a significant negative correlation with XO/XDH ratio figure (1,D), XO/XDH ratio showed a significant positive correlation with uric acid and Mo figure (1,E& F) we expect the positive correlation with uric acid may be due to the parallel increasing in XO activity observed in cholelithiasis patients reflect the fact that the catabolic pathway is increased, and this supports our results in Table (1), Mo element is a component of enzymes (XO, XDH) we suggest that the reason for positive correlation with Mo.

Conclusion:

To our knowable this is the first attempt to measure XO and XD activities in cholelithiasis patients. The increase in XO activity observed in cholelithiasis patients reflect the fact that the catabolic pathway of hypoxanthine and xanthine is increased, this increase may confirmed by increasing in uric acid levels. The results of our study show the highest XO/XDH ratio in cholelithiasis patients which confirm the idea of increase the rate of conversion of XDH to XO in this pathogenic condition, in parallel the free radical production increased and so the oxidative stress increase. XO activity as significantly increases so it may be used as diagnostic tool for this disease.

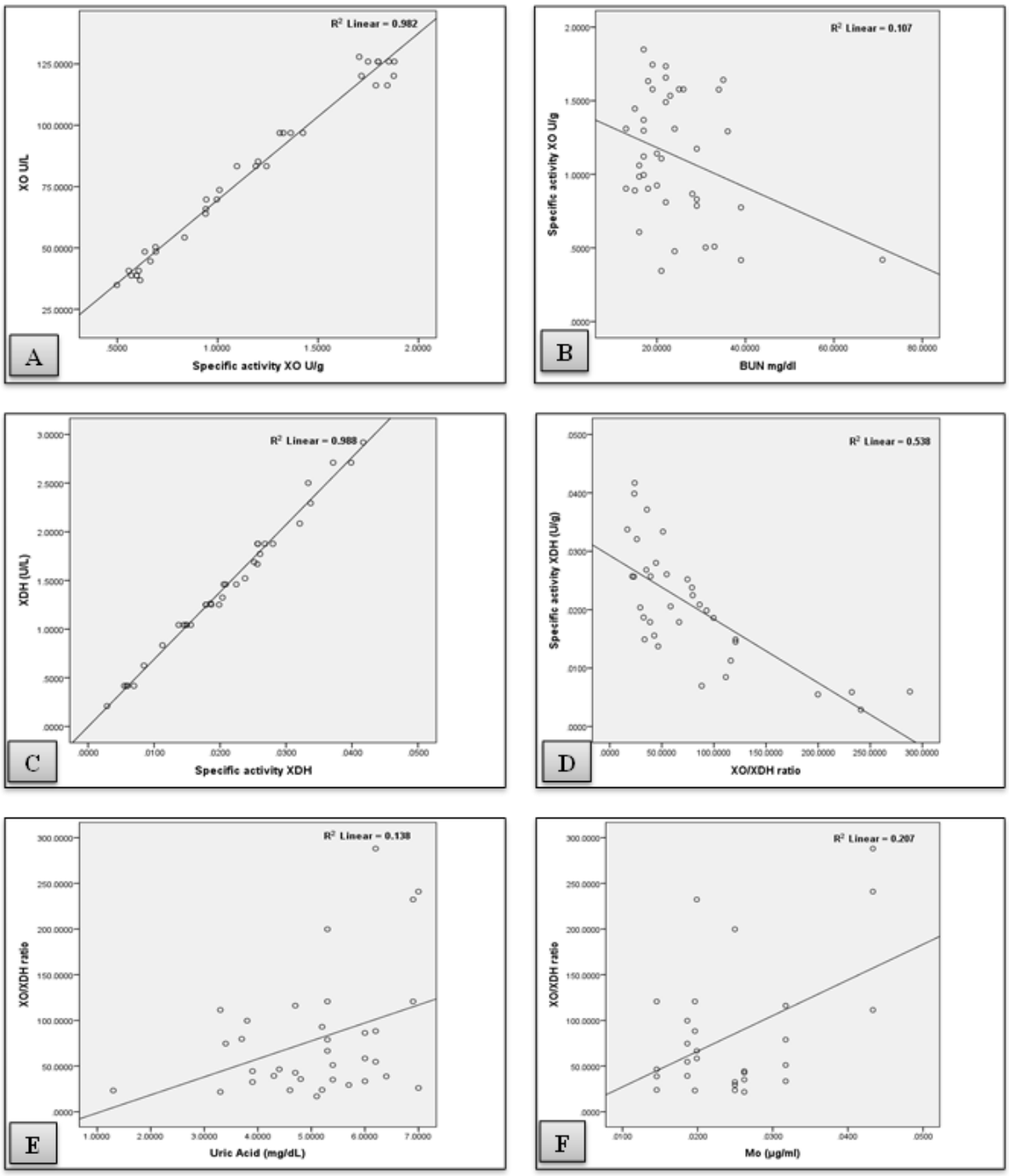

Fig. 1: Correlations between (A) XO with S.A. XO, (B) S.A. XO with BUN, (C) XDH with S.A. XDH, (D) S.A. XDH with XO/XDH ratio, (E) XO/XDH ratio with Uric Acid and (F) XO/XDH ratio with Mo.

ACKNOWLEDGMENTS

The authors are grateful to Baghdad Medical City Hospital administration and in particular the gastrointestinal tract and liver disease hospital for help us to collect blood samples and also like to thank the patients, who were very helpful in giving information and collection of samples.

REFERENCES

[1] James, S., and S.F. Dolly Anna, 2011. Sherloks disease of the liver and biliary system. 12th ed., 264.
[2] Sun, H., H. Tang and S. Jiang, 2009. Gender and metabolic differences of gallstone diseases. World J. Gastroenterol., 15: 1886-1891.

[3] Brunicardi, F Charles; Andersen, K. Dana, *et al*., 2014. Schwartz's Principles of Surgery.Tenth Edition, MD, FACS, 1270.
[4] Marschall, H-U, and C. Einarsson, 2007. Gallstone disease. Karolinska University Hospital, Huddinge, Stockholm, Sweden, J Intern Med., 261: 529-542.
[5] Jensen, 2011. Post cholecystectomy syndrome. Omaha, Nebraska: Medscape. Retrieved, pp: 01-20.
[6] Heuman, D.M., A.A. Mihas and J. Allen, 2010. Cholelithiasis. Omaha, Nebraska: Retrieved.
[7] Al-Atabi, M., S.B Chin and X.Y. Luo, 2010. Experimental investigation of the flow of bile in patient specific cystic duct models. SB Chin - Journal of biomechanical engineering.
[8] National Health Service, 2010. Gallstones- Treatment. NHS Choices: Health A-Z - Conditions and treatments. London: National Health Service, pp: 11-06.
[9] Meneshian, A., and G.B. Bulkley, 2002. The physiology of endothelial xanthine oxidase.From urate catabolism to reperfusion injury to inflammatory signal transduction. Microcirculation, 9: 162-175.
[10] Harrison, R., 2002. Structure and function of xanthine oxidoreductase. Free Radic. Biol. Med., 33: 774-797.
[11] Voet, D. and J.G. Voet, 2011. Biochemistry. John Wiley & Sons, 4th ed.
[12] Kasper, D., A. Fauci, S. Hauser, *et al.,* 2001. Harrison's Principles of internal medicine. 15th ed., McGraw-Hill Companies Inc. Medical Publishing Divis USA.
[13] Ackermann, E., and A.S. Brill, 1974. Xanthine oxidase activity. In Methods of Enzymatic Analysis, Ed. Bergmeyer H.U., 2nd ed., Academic Press, Inc., U.S.A., pp: 521-522.
[14] Fried, R., and L.W. Fried, 1974. Xanthine oxidase (xanthine dehydrogenase). In Methods of enzymatic analysis, Ed. Bergmeyer H. U., second ed., Academic Press, Inc. U.S.A., pp: 644-649.
[15] Schultz, A., 1984. Uric acid. Kaplan A Clin Chem, The C.V. Mosby Co., St Louis. Toronto. Princeton 1261-1266 and 418.
[16] Gerardo, J., L. Marianne and Y. Leonisa, 2011. Determination of the Sensitivity Range of Biuret Test for Undergraduate Biochemistry Experiments. E-JST, 6(5): 77-83.
[17] Moaed, O., and Al-Gazally, 2007. Adenosine deaminase, AMP deaminase, 5`-Nucleotidase, Xanthine oxidase and Xanthine dehydrogenase Activities in Prostate Cancer Patients. 1(3).
[18] Kasper, D.L., E. Braunwald, 2008. Harrison's Principles of Internal Medicine. New York, McGraw-Hill., 156.
[19] Chang, Y.C., F.W. Lee and C.S. Chen, 2007. Structure-activity relationship of C6-C3 phenylpropanoids on xanthine oxidase-inhibiting and free radical-scavenging activities.Free Radic Biol Med., 43(11): 1541-51.
[20] Higgins, P., J. Dawson and M. Walters, 2009. The potential for xanthine oxidase inhibition in the prevention and treatment of cardiovascular and cerebrovascular disease. Cardiovasc Psychiatry Neurol, pp; 282059.
[21] Raghuvanshi, R., and A. Kaul, 2007. Xanthine oxidase as a marker of myocardial infarction.Indian J. Clin Biochem., 22(2): 90-92.
[22] Dopp, J.M. and N.R. Philippi, 2011. Xanthine oxidase inhibition attenuates endothelial dysfunction caused by chronic intermittent hypoxia in rats. Respiration, 82(5): 458-467.
[23] Nomura, J., N. Busso and A. Ives, 2013. Febuxostat, an inhibitor of xanthine oxidase, suppresses lipopolysaccharide-induced MCP-1 production via MAPK phosphatase-1-mediated inactivation of JNK. 8(9): 75527.
[24] Cardiovascular Psychiatry and Neurology, 2009. Article ID 282059, 9 pages http://dx.doi.org/10.1155/2009/282059 Review Article.
[25] Enomoto, A., H. Kimura and A. Chairoungdua, 2002. Molecular identification of a renal urate-anion exchanger that regulates blood urate levels.Nature. 417(6887): 447-452.
[26] Mitchell, and C.H. Phillip, 2007. Overview of Environment Database. International Molybdenum Association. Archived from the original, pp: 10-18.
[27] Mendel, R.R., F. Bittner, 2006. Cell biology of molybdenum. Biochimica et BiophysicaActa., 1763(7): 621-635.
[28] Yee, Gereon M., B. Tolman, William, 2015. Transition Metal Complexes and the Activation of Dioxygen. In Kroneck, Peter M. H.; Sosa Torres, Martha E. *Sustaining Life on Planet Earth: Metalloenzymes Mastering Dioxygen and Other Chewy Gases*. Metal Ions in Life Sciences 15. Springer. pp: 131-204.
[29] Qian, Z.M., and Y. Ke, 2001. Rethinking the role of ceruloplasmin in brain iron metabolism. Brain Res Rev., 35: 287-294.