بسم الله الرحمن الرحيم

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تم رفع هذه الرسالة بواسطة / سلوي محمود عقل

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

ملاحظات: لا يوجد

جامعة القاهرة

كلية الطب البيطري

قسم الكيمياء الحيوية وكيمياء التغذية

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عنوان الموضوع : التحليل الجينى لانزيم االزانثين اوكسديز فى الفئران المستحدث فيها ارتفاع حمض البوليك

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المستخلص العربى

"التحليل الجينى لانزيم الزانثين اوكسديز فى الفئران المستحدث فيها ارتفاع حمض البوليك"

أجريت هذه الدراسة لمعرفة التغيرات التي تحدث في التعبير الجيني لإنزيم أوكسيديز الزانثين اثناء ارتفاع حمض البوليك والتاثيرالمعالج لبعض المصادر النباتيه الطبيعية الآمنة علي ارتفاع حمض البوليك فى الفئران.

لتحقيق هذا الغرض ، تم تصنيف ٥٠ فأر من ذكور الفئران :

نوع ألبينو إلى ٥ مجموعات متساوية على النحو التالي

١-المجموعة الضابطة -٢مجموعة ارتفاع حمض بوليك الدم- ٣- مجموعة ارتفاع حمض بوليك الدم المعالجة بالفيبوكسوستات

4-مجموعة ارتفاع حمض بوليك الدم المعالجة بالجنكه بيلوبا.٥--مجموعة ارتفاع حمض بوليك الدم المعالجة بعصير البصل

في نهاية التجربة (٤ أسابيع) ، تم جمع عينات الدم وفصل المصل وتحليلها لتحديد أنشطة انزيمات الزانثين أوكسيديز ، الالانين امينو ترانسفيريز، الاسبارتيت امينو ترانسفيريز ، الفوسفاتيز القاعدى وتركيزات حمض البوليك واليوريا والكرياتينين و البروتين الكلي ،عينات الكبد والكلى قسمت الي ثلاثة اجزاء الجزء الأول لتقدير محتوى مادة المالون داي الدهيد و الجلوتاثيون المختزل وحمض البوليك ونشاط ازيمى السوبر اوكسيد ديسميوتيز والجلوتاثيون بيراوكسيديز ،الجزء الثاني للفحص التشريحي المرضي و الجزء الثالث (من الكبد فقط) لدراسة التعبير الجيني لانزيم الزانثين أوكسيديز ويمكن تلخيصها على النحو التالي:

أوكسونات البوتاسيوم سببت ارتفاع حمض البوليك في الدم وزيادة قليلة للتعبير الجيني لانزيم الزانثين أوكسيديز ، نشاط انزيم الزانثين أوكسيديز و حمض البوليك في الدم والانسجة واليوريا والكرياتينين و البروتين الكلي . كما احدثت تغيرات مرضية في انسجة الكبد والكلى بدرجات متفاوتة. .

الفيبوكسوستات سبب نقص في التعبير الجيني لانزيم الزانثين أوكسيديز وتركيزحمض البوليك في المصل والأنسجة واليوريا والكرياتينين، كل - من الجنكه بلوبا وعصير البصل قلل التعبير الجيني لانزيم الزانثين أوكسيديز (قليلاً) ، ونشاط انزيم الزانثين أوكسيديز، وتركيزات حمض البوليك في الدم والانسجة واليوريا والكرياتينين،. كما أنه ادي الي تحسن في التغيرات المرضية التي حدثت فى انسجة الكبد والكلى.

استخلصت من نتائج هذه الدراسة ان مستخلص اوراق نبات الجنكة ومحلول عصير البصل الاصفر المصري تعتبر مصادر غذائية فعالة كمضادات طبيعية للاكسده وكمثبيطة لانزيم الزانثين اوكسيديز ويمكن استخدامها علاجيا في حالات الاصابة بمرض النقرس وحالات ارتفاع حمض البوليك.

الكلمات الدالة: ارتفاع حمض البوليك في الدم ، الفيبوكسوستات ، عصير البصل ،جنكة بيلوبا ، التعبير الجيني لانزيم الزانسين اوكسيديز ، انزيم الزانسين اوكسيديز ، حمض البوليك

جامعة القاهرة
كلية الطب البيطري
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التحليل الجينى لانزيم الزانثين اوكسديز فى الفئران المستحدث فيها ارتفاع حمض البوليك

تقدم

رسالة مقدمة من

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بكالوريوس العلوم الطبية التطبيقية –جامعة ٦ اكتوبر -٢٠٠٤

ماجستير الكيمياء الحيوية وكيمياء التغذية ٢٠١٠

(جامعة القاهرة)

لنيل درجة الدكتوراة في العلوم البيطرية البيطرية

(الكيمياء الحيوية وكيمياء التغذية)

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مدرس الكيمياء الحيوية وكيمياء التغذية
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I- Introduction

Gout is a condition that appears as a range of clinical and pathological characteristics based on an excess of uric acid in the body. Acute episodic arthritis is the most common form of the illness. One or more joints may become inflamed due to hyperuricemia (serum-urate level more than 6.8 mg/dL) **(Arromdee et al., 2002 and Lawrence et al., 2008).**

There are several clinical and pathological symptoms associated with Gout, which is caused by an excessive amount of Uric Acid in the blood stream. When the condition first manifests, it is often referred to as acute episodic arthritis. If you suffer from gout, you may also experience persistent arthritis in one or more joints with hyperuricemia **(Singh & Strand ., 2008).**

Glaucoma (gout) is the most frequent type of inflamed arthritis **(Rome and Mcnair., 2015)**. Most of the clinical and pathological characteristics of gout are caused by tissue deposition of monosodium urate (MSU) crystals in supersaturated extracellular fluids of the joint and some other locations. The MSU crystals are mostly located within the joints and surrounding tissues, including the periarticular and bursal tissues, as well as the bone, auricular, and skin **(Khanna et al., 2012).**

The start of gout, according to tradition, occurs between the ages of 40 and 60. An growing number of men suffer with gout, which has risen to become the most prevalent inflammatory arthritis among males throughout the world **(Annemans et al., 2008).** In the premenopausal era, women are protected from gout by female sex hormones' uricosuric action **(Zhu et al., 2011).**

It was shown in a recent qualitative study that those with chronic gout were less productive at work because of the gout **(Lindsay et al., 2011).**

As part of the xanthine oxidoreductase family of enzymes, xanthine oxidase is also known as xanthine dehydrogenase Purine catabolism is aided by xanthine oxidase and xanthine dehydrogenase enzymes in some animals and humans. This group of enzymes catalyzes the oxidation of hypoxanthine to xanthine and xanthine to uric acid. By catabolizing purines, xanthine oxidase creates reactive oxygen species (**Ardan et al., 2004 and Hille et al., 2014)**

Oxonic acid is an inhibitor of uricase enzyme, increasing uric acid level, so its potassium salt (potassium oxonate) can be used to induce experimental hyperuricemia. **(Shirasaka., 1993).** Hyperuricemia (HUA) in rats was caused by potassium oxonate (a selectively competitive uricase inhibitor) **(Tang et al., 2017)**

Inhibition of xanthine oxidation by Febuxostat is non-purine-selective (non-purine-specific). The molybdenum pterin center, which is the active site of xanthine oxidase, is non-competitively blocked by the drug. Foluxostat is used to treat persistent gout and hyperuricemia. For more information see **(Love et al., 2010).** There are structural differences between the two drugs. It is more selective and powerful than allopurinol and has no impact on other enzymes involved in the purine or the pyrimidine metabolism. Febuxostat is a non-steroidal anti-inflammatory drug (NSAID). **(Horiuchi et al., 1999)**

As the oldest tree species in the world, Gingko biloba is a Chinese tree with a 200 million-year history. Leaf extracts of Gingko biloba are the most popular phytomedicines in Europe, where they are used to treat the symptoms of vascular dementia, **(Sierpina et al., 2003) ,** elevation of uric acids in induced hyperuricemic rats (**Elatrash et al., 2015**). Ginkgo biloba has smooth, natural and relatively safe effects in reducing serum uric acid level **(Luma & Imad., 2019).**

When given to hyperuricemic rats, onions can lower their blood uric acid levels, but they have little or no impact on normal animals. This can be seen as a benefit for the onion. Allopurinol's side-effects might be minimized by using onion as a viable replacement, or at least in combination therapy, as onion is a frequent component of most people's diets across the world (**Haidari et al., 2008).**

Aim of the work

This study has been planned to investigate the changes which may be occur in the expression of xanthine oxidase gene during hyperuricemia. Also, to investigate the hyperuricemic-ameliorating effect of some natural plants (Ginkgo biloba and onion) in comparison with an anti-hyperuricemic drug (febuxostat,).

In order to realize this purpose, the plane will comprise the following items:

I- Induction of an experimental hyperuricemic condition through injection of potassium oxonate in rats.

II- Treatment trial by administration of febuxostat, Ginkgo biloba leaf extract and onion juice into hyperuricemic rats.

III- Studying changes in the expression of xanthine oxidase gene in liver.

IV- Estimating changes in xanthine oxidase activity in serum.

V- Investigating the oxidative stress condition by estimation of changes in oxidative and antioxidant parameters in liver and kidney.

VI- Determination of changes in uric acid concentration in serum, liver and kidney.

VII- Determination of changes in some liver and kidney functions.

VIII- Examination of histopathological changes in both hepatic and renal tissues.

II-Review of Literature

II. I. Gout Disease and Hyperuricemia:

According to reports, gout affects more than 2 million men and women in the United States alone, perhaps as a result of changes in eating habits during the past few decades, gouty arthritis and uric acid nephrolithiasis are commonly linked with high blood uric acid levels, leading in the deposition of urate crystals in the joints and kidneys **(Kramer and Curhan., 2002).**

Because of its severe and debilitating nature, Gout is an old kind of arthritis that's been around for ages. Some call it the "sickness of kings" because it has been wrongly associated with the type of overindulgence in food and drink that only the affluent and powerful could afford. Anyone can be affected by gout, and its risk factors differ. Most commonly, the feet, and notably the big toe, are affected by severe bouts of excruciating swelling. Unswollen areas might be hot and swollen. One in five initial episodes occurs in a joint other than the big toe, which can be affected in any joint. Food and drug triggers, as well as medications that might aid, can be avoided in order to decrease the severity of gout episodes **(Schumacher., 2015).**

Genetic implications on the relationship of hyperuricemia with gout have been studied by **Dong et al., (2017)** and **Zheng et al.,(2018)** A strong and linear connection was found between these two combinations, suggesting a possible correlation between this variation and mRNA expression. Because of this gout might be affected by the expression.

Crystal deposition disease (MSU) is one of the most prevalent adult rheumatic illnesses **(Doherty., 2009)**. It is caused by the development of monosodium urate crystals (MSU). Increased prevalence of hyperuricemia-related comorbidities such as hypertension, obesity, metabolic syndrome, type 2 diabetes, and chronic renal disease have contributed to the increase in gout prevalence over the past few decades (CKD). Some dietary changes and the extensive use of thiazide and loop diuretics for cardiovascular illnesses are also contributing to the rise in gout prevalence.

In hyperuricemia, which persists at a serum saturation of 6.8 mg/dl, urates are deposited on the articular cartilage, causing gout. Even with high levels of serum uric acid, very few

people get gout. This highlights the relevance of other variables in crystal formation **(Conway and Schwartez., 2009).**

It has been shown that elderly women are at greater risk of developing gout because they have higher amounts of urate acid in their blood. Men have higher urate acid levels than women and gout is more common in older women **(Wallace et al .,2004)**.

Atypical gout symptoms can occur in women; females are generally a decade older than men when they first get gout and are less likely to have metatarsophalangeal involvement; instead, polyarticular gout affects the ankles or the joints of the fingers and upper limbs **(Dirken-Heuensfeldt et al., 2010).**

Nevertheless, other variables in the joint can affect urate's solubility in joint fluids. There are several elements to consider including temperature, pH cations, dehydration, and the presence of nucleating substances such as non-aggregated proteoglycans, insoluble collagens, and chondroitin sulfate **(Gill and Dieppe., 1991).** The risk of gout linked with an increase in serum urate level may be affected by variations in these variables. The predisposition to gout in the first metatarsal phalangeal joint may also be explained by these variables (a peripheral joint with a lower temperature) and degenerative joints with nucleating debris (osteoarthritis), nocturnal onset of discomfort due to intra-articular dehydration **(Lin et al . , 2000) .**

More than 90 percent of instances of hyperuricemia are due to poor renal excretion of urate, or a combination of these two processes (decrease renal exceretion and over production of uric acid) **(Conway and Schwartez., 2009).**

In extracellular areas, such as the joint or soft tissue, uric acid concentrations of 6.8 mg/dL surpass its solubility. However, for a variety of reasons, urate crystals may not always induce an inflammatory response. The chance of developing symptomatic hyperuricemic is directly related to the level of serum urate elevation **(Samuel et al . , 2009)**

When monosodium urate crystals accumulate in and around joints, gout is a genetic metabolic disease. Rheumatoid arthritis episodes are caused by these crystals on a regular basis In the U.S., gout is one of the most prevalent causes. There are two main types of gout. Ninety percent of all cases are primary gout. A secondary gout condition occurs in 10% of cases, where the elevated uric acid is caused by another condition (e.g., excessive breakdown

of cells or renal disease). Inhibitors of uric acid excretion include diuretics used to treat hypertension and low-dose aspirin Primary idiopathic gout hyperuricemia has two causes: Increased uric acid synthesis (majority of cases)

Low excretory capacity (30 percent of cases) may lead to nephrolithiasis (uric acid crystallization) and kidney disease. Usually, the exact metabolic abnormality is unclear, but the condition can be controlled in the vast majority of cases **(Joseph et al., 2016)**

On the other hand, according to **(Campion et al ., 1987)**, among patients with low levels of serum Urate, the yearly incidence of Gouty Arthritis is just 0.1 percent.

Individuals with serum urate levels more than 9.0mg/dL had a cumulative incidence rate of 22% of gouty arthritis after five years of follow-up, indicating that many patients with high blood uric acid levels are not impacted by gout. Whilst the severity of hyperuricemia is associated with an increased chance for developing the disease, hyperuricemia itself can continue for years without symptoms. It is thus not recommended to treat asymptomatic hyperuricemia on a purely empirical basis

In the bloodstream, uric acid is produced through the metabolism of purine nucleic acids, which can be found in red meat, beer, shellfish, and yeast extracts as a result of the work of **(Hochberg and Silman . , 2008)**.

One of the enzymes involved in purine catabolism is xanthine Pyrophosphate Synthetase (PRPS), another is Hypoxanthin-Guanine Phosphoryl Transferase (HRPT), and the third is Xanthin Oxylase. For further information, see **(Kassmatis et al., (2009).**

Some individuals develop hyperuricemic syndromes due to a lack of HRPT or excessive PRPS activity. While other animal species do express uricase, humans and other primates do not. Consequently, uric acid is the end result of purine catabolism in humans and is eliminated in urine and, to a lesser extent, in feces For hyperuricemia, the cornerstones of treatment are xanthine-oxidase inhibitors, and medicines that enhance renal uric acid excretion (uricosurics). It is important to note that **Samuel et al. (2009),** state that:

Serum urate levels may be reduced by altering food and lifestyle. Purine-rich meals such as red or organ meats, seafood, and beer should be avoided by these individuals. Even weight loss and exercise can help reduce urate levels **(Dessein and Shipton., 2000)**

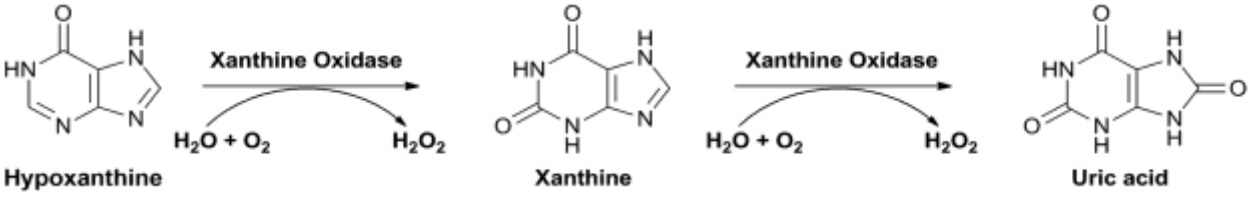
Gout is an uncommon disease caused by enzyme abnormalities in purine metabolism. Gout in males is typically caused by a high hereditary predisposition, even though the genetic basis is unclear. Recent research has focused on genes that regulate the transfer of the salty substance, known as urate. Urate transporter 1

(URAT1), encoded by SLC22A12, is a member of the organic anion transporter family and plays a crucial role in regulating uric acid reabsorption from the renal tubules. Hyperuricemia has been linked to a polymorphism of this gene**. (Graessler et al. , 2006).**

II.II. Xanthine Oxidase Enzyme:

II. II. I. Xanthine oxidase and uric acid synthesis:

"The enzyme Xanthine Oxidase" (EC 1.17.3.2) catalyzes hypoxanthine and xanthine oxidation to produce uric acid, which is the rate-limiting step in the production of uric acid. By inhibiting uric acid production, XO inhibitors can be powerful therapeutic medicines for the prevention of hyperuricemia. Clinically, the XO inhibitor Allopurinol treats gout. A hypersensitive response or Stevens-Johnson syndrome might occur. Renal damage and liver necrosis are also possible adverse effects. Many XO inhibitors have been identified and described from plants, and it is expected that they can be utilized as alternatives to allopurinol since they have less side effects **(Zanabaatar et al. , 2010).**



Synthesis of uric acid

(Ming, et al,2012)

About 150-kilodaltons of molecular mass make up the Xanthine dehydrogenase (XDH) homodimer. First, the thiol group oxidation occurs, which may be reversed by treating the enzyme with thiol reagents **(Della and Stirpe . , 1972).**

It is hypoxanthine and xanthine that catalyze the last two stages of purine catabolism in humans, producing uric acid from hypoxanthine and xanthine, respectively. However, the enzyme can be transformed into both a NAD+-dependent dehydrogenase and an oxidase both in vitro and in vivo. As an electron acceptor, oxidase produces large quantities of reactive oxygen metabolites under specific situations, such as during tissue reoxygenation following hypoxia (see below for more information) **(McCord ., 1985).**

Each subunit is then broken down into an approximately 20-kilodalpha fragment, which is then irreversibly converted. This conversion occurs during purification procedures, unless the enzyme is protected by protease inhibitors, which prevents the enzyme from being broken down into its constituent subunits. Even with such measures, tissue preparations generally include 10–15 percent of their total XDHXO activity in the oxidase form. The dehydrogenase form does not have a complete oxidase activity, and this cannot be determined with certainty. XDH(hXDH)XO, which encodes a human protein, has been located at a single locus on 2p22. This may be due to variations in the nucleotide probe sequences employed to detect hXDHXO mRNA expression as well as variances in the cDNA sequences themselves **(Mika and Kari ., 1996)**.

This enzyme is similar to aldehyde-oxidizing enzyme in terms of protein structure and prosthetic group composition, but it has a different preference for substrate. Due to a mutation of two amino acid residues located at its active site,

purine substrate preference is changed from XOR type to AO type **(Yuichiro et al ., 2015) .**

Because it is a rate-limiting enzyme, Xanthine Oxidoreductase (XOR) has two forms: xanthine dehydrogenase and xanthine oxidase. In humans, the end result of purine metabolism is uric acid, which has a strong antioxidant effect. To compensate for the absence of ascorbic acid in hominoids, the uricase gene has been mutated to pseudogene status, leading to an increase in the antioxidant capacity of uric acid **(Furuhashi., 2020)**.

II. II. II.Xanthine Oxidase Induces Oxidative Stress:

Humans and mice with infectious hepatitis have XDH/XO activity up to 50 times normal (**Giler et al., 1975**). A study by **(Akaike et al., 1990)** suggests that XDH/XO has a major pathological function in viral diseases. In addition, XO-catalyzed production of reactive O2 species contributes significantly to influenza-related mortality.

Disturbances in energy metabolism, according to **Boda et al., (1984)**, can lead to the breakdown of ATP and increase XO-mediated damage In the pathophysiology of newborn respiratory distress, XO-derived reactive O2 species have been postulated as key mediators.

There are several ways to transform the human enzyme (XDH) into the enzyme xanthine xanthine (XO), which generates both superoxygen and hydrogen peroxide in roughly a 1:3 molar ratio, depending on the circumstances. For example, tryptophan 335, a key amino acid cluster in the XDH-to-XO transition, was substituted with alanine, and leucine was replaced with phenylalanine 336, which regulates FAD's redox potential through stacking interactions with the flavin cofactor. There is a striking resemblance between the bovine XO form and this mutant's active site loop chain trace. Thus, XDH and XO versions of the mutant are in an equilibrium that favors the XO form, according to these data **(Nishino et al ., 2008)**

With various physiological activities linked with antioxidant (uric acid: Uric acid) and many reactive oxygen species (H2O2), Xanthine Oxidase (XO) is one of the significant regulators of the cellular redox potential involved in organogenesis and development. These studies have helped researchers better grasp the possible role that this enzyme may have in these tissues' development. Embryos from broiler and layer chickens differed significantly in their expression of XOR gene, their XO activity, and their UA content **(Naseri et al.,2017)**

Animal species and tissues have a large amount of the molybdenum-iron-sulfur flavin hydroxylase, Xanthine dehydrogenase (XDH). A range of nitrogenous molecules, including purines, pyrimidines, and pteridines, are oxidized by the enzyme, enzyme that slows down nucleic acid decomposition by channeling purines to the final oxidation step. Changes the NAD-dependent dehydrogenase form (XDH) to the O2-dependent oxidase form