Effects Of *Ginkgo biloba* Versus Celecoxib On The Levels Of Oxidative Stress And Matrix Metalloproteinase-1 In Patients With Knee Osteoarthritis

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Abstract

To investigate the effects of *Ginkgo biloba* (GB) and Celecoxib on the parameters of oxidative stress (OS) and matrix metalloproteinase-1(MMP-1) concentration and the markers of inflammation in patients with knee osteoarthritis (KOA).
To achieve the aim of this study, a randomized control trial(RCT) was adopted. A total of 80 patients with KOA were recruited and investigated for parameters of OS which included: malondialdehyde (MDA), total antioxidant status (TAS) and serum MMP-1 concentration, in addition to measuring the markers of inflammation such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). The patients were divided into 2 groups namely, the GB plus celecoxib group which consisted of 40 patients(with mean ± SD= 51.10 ± 9.78) and the Celecoxib group which consisted of 40 patients(with mean ± SD=51.47±6.26). The patient groups were followed- up for 8 weeks during which the parameters under study were measured before starting therapies and at the end of the follow-up period using commercially available kits. The patient groups were compared with a control group consisted of 40 apparently healthy subjects. By comparing the concentrations of MDA,TAS and MMP-1 in patients with KOA before and after therapy , there was a highly significant decrease (p<0.001) in serum MDA concentration and a significant decrease (p=0.02) in MMP-1 levels with a highly significant increase (p<0.001) in TAS values after two months use of GB plus celecoxib and celecoxib alone, but with results in favor of the former group. GB plus celecoxib seemed to produce a highly significant decrease (p<0.001) in CRP and ESR compared to celecoxib after two months of therapy. The use of GB plus celecoxib and Celecoxib alone for 8 weeks in KOA patients have beneficial effects on the parameters of OS, MMP-1 and inflammatory markers.

Keywords: Knee osteoarthritis, Ginkgo biloba , Celecoxib, Oxidative stress, Matrix metalloproteinase.

تأثير الجنكوبايلوبا مقابل السيليكوكسب على مستويات إجهاد الأكسدة والبروتيناز الفلزي المطرسي الأول عند مرضى سوفان الركبة

الخلاصة

لدراسة تأثير الجنكوبايلوبا و السيليكوكسب على معايير اجهاد الاكسدة وتركيز البروتيناز الفلزي المطرسي الأول ودلائل الالتهاب عند مرضى سوفان الركبة . لتحقيق أهداف هذه الدراسة ,تم اعتماد تصميم محاولة عشوائية ضابطة .تم اشراك ثمانين مريضا مصابا بسوفان الركبة لغرض قياس معايير إجهاد الأكسدة والتي

شملت على المالوندايلديهايد ,حالة مضادات الأكسدة الكلية وتركيز البروتيناز الفلزي المطرسي الأول في مصل الدم ,بالإضافة الى قياس دلائل الالتهاب مثل البروتين التفاعلي نوع ج ومعدل تراكم الكريات الحمراء .تم تقسيم المرضى إلى مجموعتين متساويتين :مجموعة الجنكوبايلوبا مع السيليكوكسب و مجموعة السيليكوكسب ضمت كل واحدة على40 مريضا .تمت متابعة المرضى لمدة 8 أسابيع ,أجريت خلالها قياس المعايير أعلاه قبل وبعد انتهاء فترة المتابعة بواسطة عدة عمل متوفرة في السوق المحلية .تم مقارنة مجموعة المرضى مع مجموعة ضابطة اشتملت على 40 شخصا سليما ظاهريا من خلال مقارنة نتائج المالوندايلديهايد وحالة مضادات الأكسدة الكلية و البروتيناز الفلزي المطرسي الاول عند مرضى سوفان الركبة ,كان هناك نقصا معنويا عاليا($p<0.001$) في تركيز المالوندايلديهايد في مصل الدم مع نقصان معنوي ($p=0.002$) في مستوى البروتيناز الفلزي المطرسي الاول مع زيادة معنوية عالية ($p<0.001$) في قيم حالة مضادات الأكسدة الكلية بعد شهرين من استعمال الجنكوبايلوبا مع السيليكوكسب والسيليكوكسب لوحده مع تاثير مفضل لصالح المجموعةالاولى.اظهر استعمال الجنكوبايلوبا مع السيليكوكسب نقصا معنويا عاليا ($p<0.001$) في البروتين التفاعلي نوع ج ومعدل تراكم الكريات الحمراء بالمقارنة مع استعمال السيليكوكسب بعد شهرين من العلاج. لاستعمال الجنكوبايلوبا مع السيليكوكسب والسيليكوكسب لوحده لمدة 8 أسابيع عند مرضى سوفان الركبة تاثيرات مفيدة على معايير اجهاد الاكسدة و البروتيناز الفلزي المطرسي الاول ودلائل الالتهاب.

كلمات الدلالة :سوفان الركبة ,جنكوبايلوبا , سيليكوكسب , اجهاد الاكسدة , البروتيناز الفلزي المطرسي الاول ,دلائل الالتهاب.

Introduction

Osteoarthritis is the most prevalent rheumatological disorder and frequently affects the weight-bearing joints such as hips and knees (Külcü,2010). It is the most common joint disease, characterized by pain , inflammation, and stiffness due to degeneration of articular cartilage (Shiller, 2002). Cartilage degeneration and inflammation stimulate new bone (spur) formation around the joint (Ciombor *et al*., 2003) that result in chronic disease and disability with advanced age and seriously alter the quality of life (Woo *et al*., 2004). Symptomatic Knee OA occurs in 13% of people aged ≥60 years, and by 2020, it is estimated that the number of people with OA will have doubled (Hunter, 2011). The risk factors for OA development are well –known, and they are broadly dividable into those that are constitutional or genetic and those that are local and driven by biomechanical elements such as excessive joint usage (Mounach *et al*., 2008). Obesity and joint injury are the two major modifiable factors. Age and sex are most powerful risk factors (Blagojevic *et al*., 2010; Yoo,2010). Although in the past OA was frequently regarded as a noninflammatory form of arthritis , there is now a strong evidence that proinflammatory cytokines derived both from chondrocytes and from synovium are predominant factors in the cartilage destruction associated with OA (Ray *et al*., 2003). Data from literature indicates a link between free radical burden and OA pathogenesis mediating local tissue reactions between the joint compartment . Hence ,OS is likely not only to promote cartilage destruction but also be involved in inflammative transformation , promoting the transition from clinically –silent cartilage destruction to apparent OA. The interrelationship between OS and OA etiology might provide a novel approach to the comprehension and therefore; modification of disease progression and symptom control (Alcaraz *et al*., 2010; Ziskoven *et al*., 2010). MMPs are believed to be responsible for the destruction of connective tissues at sites of chronic inflammation such as arthritis (Lu and Wahl, 2005). MMPs are family of zinc-binding, calcium- dependant endopeptidases that degrade

components of extracellular matrix (ECM), present at low levels and play important roles in many physiological processes (Brinckerhoff and Matrisian, 2002). MMP-1 , also known as collagenase 1 , is one of the major enzymes that specifically degrades type Π collagen, it is expressed in articular chondrocytes and synthesis of this protein is induced at high levels in OA (Ray *et al*., 2003; Prado and Selman, 2005). A redox-component to age-associated increase in MMP-1 has been established. The involvement of ROS in age-associated MMP expression provide a mechanistic link between free radical theory of aging and many age-associated degenerative disease such as OA (Fisher *et al*., 2009). Much of the therapeutic effort in osteoarthritis has rightly concentrated on effort to limit the formation of inflammatory mediators such as prostaglandins and leukotrienes with anti-inflammatory agents (Reijman *et al*., 2005). Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used to alleviate the symptoms of OA. It appeared that COX-2 selectivity resulted in cartilage reparative properties, whereas the absence of COX-2 selectivity could even result in negative effects (Mastbergen *et al*.,2006) . The underlying principle behind the therapeutic action of the Ginkgo leaf extract (GLE) on chronic ailments has focused on its antioxidant properties. The 2 proposed mechanisms of action are (1) directly scavenging free radicals and (2) indirectly inhibiting formation of free radicals. The GLE can also enhance activities of antioxidant enzymes such as superoxide distmutase (SOD), glutathione peroxidase, catalase, and/or heme-oxygenase-1, thereby indirectly contributing as an antioxidant (Mahadevan and Park, 2008).

Patients and Methods

This study included a newly diagnosed patients with KOA**.** One-hundred patients aged 50 years and older complaining from unilateral or bilateral knee pain who fulfilled the classification criteria of idiopathic KOA of the American College of Rheumatology (ACR) were recruited in this study. Clinical and radiological criteria of idiopathic knee OA according to ACR Knee pain plus at least one of three:

Age >50 years
Stiffness <30 minutes
Crepitus
+
Osteophytes

The sensitivity and specificity of these criteria per se are 91% and 86% respectively (Klippel *et al*., 2008) . The patients who were eligible to the study started to receive either celecoxib (celex) [Alpha-Aleppo Pharmaceutical Ind, Syria] in a fixed dose of 200 mg per day in the first group that consisted of fifty patients or celecoxib plus *Ginkgo biloba* (*Ginkgo biloba*) [Adrien Gagnon, Canada] in doses of 200 mg for celecoxib and 500 mg twice daily for *Ginkgo biloba* in the second group that consisted of fifty patients. Patients in both groups were followed for two months period. Fifty apparently healthy volunteers (twenty males and thirty females, aged 50 years and older) with mean age ±SD of 50.77 4.28 years who do not complain of knee pain and have no clinical signs of asymptomatic knee OA were recruited as control (with age ,sex ,and BMI matching to the patient groups). About 10 ml of venous blood was withdrawn from OA patients prior to the initiation of any medication and after two months of the drug use. Sample from the healthy control subjects were collected and processed in the same way. The biochemical analysis were performed in the clinical

laboratory of department of Pharmacology, College of Medicine, University of Mosul using commercially available kits except for MDA which was measured by reagent methods.

Results

The patients were equally assigned to two groups, namely the *Ginkgo biloba* plus Celecoxib, and the Celecoxib ones. Forty apparently healthy subjects with no previous history and clinical presentation of KOA were served as a control group. The characteristics of the patients and controls were given in table (3.1). By comparing the values of MDA,TAS and MMP-1 in patients with KOA before and after therapy , there was a highly significant decrease (p<0.001) in serum MDA and a significant decrease (p=0.02) in MMP-1 levels with a highly significant increase (p<0.001) in TAS values after two months use of GB plus Celecoxib, as shown in table (3.2). By comparing the values of MDA,TAS and MMP-1 in patients with KOA before and after therapy, there were a significant decrease in MDA and MMP-1 levels (p=0.011 & p=0.04 respectively) and a highly significant increase (p=0.008) in TAS levels after two months use of Celecoxib, as shown in table (3.3). Table (3.4) illustrates that the use of GB plus Celecoxib and Celecoxib for two months, although they have resulted in significant decreases in MDA and MMP-1 levels (p=0.001 & p=0.02 respectively) and significant increases in TAS levels (p=0.001), GB plus Celecoxib appeared to produce significant differences with regard to its effects on the markers of oxidative stress and MMP-1as compared to Celecoxib therapy.

Discussion

Osteoarthritis is associated with greater than normal **lipid peroxidation** (LPO) and an imbalance in antioxidant status, not only in the blood, but also in several other cellular systems ,which support the role of oxidative stress (OS) in its pathogenesis (Rèdon *et al*., 2003; Grossmann, 2008). **Malondialdehyde (MDA)** is the most generally used index of LPO in the appreciation of the role of OS in disease and it is often assayed with thiobarbituric acid (TBA) procedure (Le Fèvre *et al*., 1998; Gawat *et al*.,2004; Garenova and Gadjeva, 2005), which was used in this study. The results of present study indicated higher OS in knee osteoarthritis (KOA) patients, either due to increased extent of LPO as shown by the significantly increased levels of MDA or due to decreased levels of antioxidants as shown by the significantly decreased levels of TAS, as compared with healthy control subjects. The above findings were in agreement with the study of Maneesh *et al*., (2005) who observed that osteoarthritis patients were more susceptible to oxidative damage than controls as evident from increased TBA reactive substances and decreased ascorbic acid, GSH, catalase and GPx in erythrocytes, and that of El-barbary *et al*.,(2011) and Fernandez-Moreno *et al*., (2011) who found that increased oxidative stress in and OA patients have led to compensatory changes in the levels of antioxidants and these changes provide additional protection against LPO. They concluded that these findings confirm the role of oxidative stress in the pathogenesis of and OA, and that LPO markers and antioxidants can serve as surrogate markers for disease activity. In the current study, the use of *Ginkgo biloba* (GB) plus celecoxib was associated with significant decrease in mean serum MDA levels (

from 2.90±0.72 to 2.01±1.03; *P* value=0.001) and significant increase in mean serum TAS levels (from 1.10±.30 to 1.48±0.40; *P* value=0.001) after two months of therapy, while the use of celecoxib alone was associated with significant decrease in mean serum MDA levels (from 2.55±1.01 to 2.12±0.56; *P* value=0.011) and significant increase in mean serum TAS levels (from 1.10±0.39 to 1.29±0.23; *P* value=0.008) but the values were still different from the control healthy subjects. From noticing the above results, both drugs seemed to be comparable to each other regarding their effects on mean serum MDA and TAS concentrations, but when the differences of percentage variation of MDA and TAS of GB plus celecoxib were compared with that of celecoxib alone, it was found that the use of GB plus celecoxib for two months was associated with more reduction in the mean serum MDA concentrations (-0.98± 0.24; *P* value=0.001) and more increase in mean serum TAS concentrations (+0.40± 0.08 ; *P* value= 0.001) and their effect appeared to be more favorable compared to that of celecoxib alone (-0.43± 0.16 for MDA and +0.20 ± 0.07 for TAS respectively). Furthermore, the observed decrease in the MDA levels with concomitant increase in the TAS levels after two months therapy with *Ginkgo biloba* plus celecoxib and celecoxib may give clue to the benefit obtained by KOA patients from using such drugs. These findings were consistent with a large body of data showing that reactive oxidative species, such as nitric oxide (NO) and ROS, are important in the pathogenesis of KOA. These findings suggested that local accumulation of proteins altered by the reaction between ROS and NO may be important in the pathogenesis of OA. Oxidative damage in cartilage may affect chondrocyte function through inducing telomeric DNA instability, replicative senescence and dysfunction of chondrocytes in OA cartilage, resulting in changes in cartilage homeostasis that are relevant to cartilage ageing (chondrocyte senescence) and promoting the transition from clinically silent cartilage destruction to apparent OA (Yudoh *et al*.,2005 ; Ziskoven *et al*.,2010). The free radical scavenging activity of GB extract (GBE) was studied thoroughly by many authors in human and animal models and all ascertain the antioxidant properties of it and they were in agreement with the results of the current study. One of them was that of Akiba *et al*., (1998) who found that the suppressive effect is specific on platelet aggregation stimulated by OS. Several studies investigated the molecular mechanisms of antioxidant action of CBE. Woo *et al*.,(2003) studied the effect of GBE on homocysteine-stimulated inducible NO synthase expression in macrophages and demonstrated that GBE and its terpenoids antagonized the stimulatory effect via antioxidation and attenuation of nuclear-factor k B (NFk-B) activation . Wei *et al*.,(1999) further confirmed that GBE suppresses H_2O_2-induced activation of NFk-B in bovine pulmonary endothelial cells in a concentration-dependent fashion. The ability of celecoxib to improve joint inflammation came from the fact that the OA-affected cartilage in ex vivo conditions spontaneously releases NO (which enhances cyclooxygenase activity and PGE2 production in various cell types including normal human chondrocytes) in quantities sufficient to cause cartilage damage. The importance of these findings is supported by experiments conducted in animal models of arthritis, where inhibitors of NOS ,COX-2 or NFk-B

can independently repress joint inflammation, concomitant with the attenuation of PGE2 or NO synthesis, which are in agreement with the results of present study (Amin *et al*., 1997; McKenna *et al*.,2001). **Matrix metaloproteinases (MMPs)** are expressed in OA cartilage and are thought to be involved in the degradation of cartilage extracellular matrix (ECM) (Imai *et al*., 1997).The involvement of ROS in age-associated MMP expression provides a mechanistic link between free radical theory of aging and many age-associated degenerative diseases like OA (Dasgupta *et al*., 2010). MMP-1, also known as collagenase-1, is one of the major enzymes that specifically degrades type II collagen. It is expressed in articular chondrocytes, and synthesis of this protein is induced at high levels in OA (Ray *et al*.,2003). The results of present study showed that patients with KOA have significantly higher mean serum MMP-1 concentrations in both drug groups (34.33±21.53 and 37.03±16.57, p=0.001 for GB plus celecoxib and celecoxib, respectively) as compared to mean serum MMP-1 concentrations in healthy control group (6.80±2.90). The above results were in agreement with the results of Green *et al*., (2003) and Chen *et al*., (2011) who found that in 98 patients with early untreated RA of less than 12 months duration serum MMP-1 levels were significantly greater in RA patients than in controls and serum MMP-1 levels correlate with disease activity and predict functional and radiographic outcome in early untreated RA. It has been reported that induction of MMP-1 or COX-2 and PGE2 involves the activation of NFk-B and the H_2O_2-mediated increase in cyclo-oxygenas-2(COX-2) through NFk-B activation results in additional generation of prostaglandin E2 (PGE2) that enhances MMP-1 production by a pathway involving c AMP (Lu and Wahl ,2005). In the present study, the use of both drugs for 2 months was associated with significant decrease in the mean serum concentrations of MMP-1 (from 34.33±21.53 to 24.20±12.38, p=0.02 for GB plus celecoxib and from 37.03±16.57 to 29.93±12.70, p=0.04 for celecoxib). The antiarthritic and analgesic activity of GB comes from the fact that GB directly act as an NO scavenger in addition, it inhibits NO production in LPS -activated macrophages by concomitant inhibition of induction of inducible nitric oxide synthase (iNOS) mRNA and the enzyme activity of iNOS. Thus, GB may act as a potent inhibitor of NO production under tissue-damaging inflammatory conditions, and suggest that blockage of the NO production from the macrophages that infiltrated to the inflamed site may be a possible mechanism for the therapeutic anti-inflammatory effect of GB (Lu and Wahl ,2005). The benefit of celecoxib in KOA treatment seen in this study explained by COX-2 inhibitors which inhibited the appearance of nuclear-acting prostanoid ligands, suggesting that it is produced from the COX-2 pathway (Gilroy *et al*., 1999). Recent studies showed that in addition to stimulating the PPAR-gamma receptors, these nuclear-acting prostanoid ligands inhibit the Ik-B kinase activity and thereby block the NFk-B transcription factor pathway (Rossi *et al*., 2000). Both GB and celecoxib were able to reduce MMP-1 concentration to nearly the same extent, but when the difference of percentages variation was compared for both drugs, GB seemed to produce more favorable effects in comparison to that of celecoxib (-10.13± 4.17 for GB and -7.10 ± 3.26 for celecoxib with p=0.02). The present study showed that the markers

of inflammations ,ESR and CRP were significantly elevated in both drug groups in comparison with control group. The mean concentration of CRP in the GB plus celecoxib group was 16.80± 8.40 compared with 17.40 ± 7.75 in the celecoxib group which are significantly higher(p=<0.001) than in the control group (6.67± 1.93). These findings are in agreement with the study of Wolfe,1997 who found that CRP was elevated in KOA compared to healthy individuals, and the study of Murphy *et al*.,2008 who found that systemic markers of inflammation were elevated in KOA patients. The mean concentration of ESR in the GB plus celecoxib group was 27.77 ±5.10 compared with 26.23 ± 9.47 in the celecoxib group which are significantly higher (p<0.001) than in the control group (17.43± 7.18).

Table (3.1):- The characteristics of KOA patients and control group.

Groups	Parameters	Mean SD	*P*- Value
Control	Age (Year)	**50.77± 4.28**	**0.87***
KOA patients on GB plus Celecoxib	Age (Year)	**51.10 ± 9.78**	
Control	BMI (Kg/m^2)	**27.9 ± 1.73**	**0.06***
KOA patients on GB plus Celecoxib	BMI (Kg/m^2)	**29.73 ± 4.93**	
Control	Age (Year)	**50.77± 4.28**	**0.62***
KOA patients on Celecoxib	Age (Year)	**51.47± 6.26**	
Control	BMI (Kg/m^2)	**27.9 ± 1.73**	**0.07***

KOA patients on Celecoxib	BMI (Kg/m^2)	**29.80 ± 5.30**					
Parameter		**Control**		**KOA patients on GB plus Celecoxib**		**KOA patients on Celecoxib**	
		No.	**%**	**No.**	**%**	**No.**	**%**
Sex	Male	14	35	12	30	14	35
	Female	26	65	28	70	26	65
	Total	40	100	40	100	40	100
	***P*- Value**			**0.32***		**0.26***	

* Non significant difference from control using unpaired t-test

Table (3.2):- Comparison of MDA ,TAS ,MMP-1 ,CRP & ESR between patients on GB plus Celecoxib (Before &After) therapy.

Parameters	Groups	Mean ± SD	*p*-value
MDA (µmol/L)	Before	2.90±0.72	0.001*
	After	2.01±1.03	
TAS (mmol/L)	Before	1.1±0.30	0.001*
	After	1.48±0.35	
MMP-1 (ng/L)	Before	34.33±21.53	0.02*
	After	24.20±12.38	
CRP (mg/dl)	Before	16.80± 8.40	0.01*
	After	13.27± 7.20	
ESR(ml/hr)	Before	27.77 ±5.10	0.001*
	After	20.76 ±3.43	

*Significant differences using paired t-test

Table (3.3):- Comparison of MDA ,TAS ,MMP-1, CRP &ESR in KOA patients on Celecoxib (Before & After) therapy.

Parameters	Groups	Mean ± SD	*p*-value
MDA (µmol/L)	Before	2.55±1.01	0.011*
	After	2.12±0.56	
TAS (mmol/L)	Before	1.1±0.39	0.008*
	After	1.29±0.23	
MMP-1 (ng/L)	Before	37.03±16.57	0.04*
	After	29.93±12.70	
CRP (mg/dl)	Before	17.4±7.75	0.01*
	After	15.77± 7.20	
ESR (ml/hr)	Before	26.23 ± 9.47	0.02*
	After	24.37± 4.63	

*Significant differences using paired t-test

Table(3.4):- Difference of percentage variation between KOA patients on GB plus Celecoxib and Celecoxib therapies.

Parameters	Mean SE (%variation after drug use)		*p*-value
	GB plus Celecoxib	Celecoxib	
MDA (µmol/L)	-0.98 ± 0.24	-0.43 ± 0.16	0.001
TAS (mmol/L)	+0.40 ±0.08	+0.20 ± 0.07	0.001
MMP-1(ng/L)	-10.13± 4.17	-7.10± 3.26	0.02
CRP (mg/dl)	-3.53± 1.33	-1.63 ± 0.34	0.001*
ESR (ml/hr)	-7.0 ± 1.27	-2.17 ±0.85	0.001*

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