INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 21, No. 3, p. 1-7, 2022

RESEARCH PAPER OPEN ACCESS

Protective effect of lectins extracted from *Eucalyptus globules* against LPS induced renal oxidative stress

Sana Boufeker, Ahlem Bahi, Imen Torch, Youcef Necib*

Laboratory of Microbiological Engineering and application,Faculty of Natural and Life Sciences, UniversityMentouri Brothers Constantine, Algeria

Key words: Lectins, Anti-inflammatoryactivity, *Eucalyptus globules,* Algeria.

http://dx.doi.org/10.12692/ijb/21.3.1-7 Article published on September 03, 2022

Abstract

This study will highlight the potential protective role of a new lectin extracted from *Eucalyptus globules* in lipopolysaccharide-induced renal inflammation using kidney tissue lipid peroxidation, GSH levels, SOD, GSH-Px, and catalase activities to assess the effects of lectin of *Eucalyplusglobuls* on LPS-induced oxidative and renal stress. PS administration led to a significantly reduced GSH level, SOD, GSH-Px, and catalase activity in kidney tissue, as well as higher lipid peroxidation levels .in addition, LPS therapy resulted in a significant increase in kidney weight and a drop in overall weight. Treatment with lectin of *Eucalyplus globuls* significantly moderated the damaging consequences of LPS on oxidative stress biomarkers and reduced the histological alterations in the kidney produced byLPS. Our findings suggest that the lectin of *Eucalyplus globuls* may protect rats from nephrotoxicity and oxidative stress caused by LPS.

* **Corresponding Author:** Youcef Necib ✉ youcefnecib@yahoo.fr

Introduction

Oxidative stress is thought to be a major pathogenic mechanism that plays a role in the onset and progression of kidney injury. Antioxidants are a sensible curative strategy for preventing and curing oxidative stress-related kidney disorders; Antioxidant and free radical scavenging properties are common in natural antioxidants found in edible and medicinal plants, as well as anti-inflammatory properties, which are thought to constitute the foundation for numerous bioactivities and health (Sha *et al*, 2015). Several pathogenic processes have been identified as significant participants in the pathogenesis of kidney injury, including oxidative stress, tubular epithelial cell death, and Several enzymes (e.g., superoxide dismutase (SOD), catalase, and glutathione peroxidase) and non-enzymatic substances (e.g., tocopherol, vitamin E, beta-carotene, ascorbate, and glutathione (GSH)) protect against ROS **[**Morrell *et al*, 2014; Rapa *et al*, 2020**).** Lipopolysaccharide (LPS), an endotoxin produced by gram-negative bacteria, interacts with toll-like receptor 4 (TLR4) to activate renal tubular epithelial cells and pro-inflammatory cells [Morrell *et al*, 2014, Peerapornratana *et al*, 2019]. Active compounds derived from natural products have recently been highlighted as potential novel therapeutic candidates for kideny stress) [Rapa *et al*, 2020, Chen *et al*, 2018) .EC is one of the most medicinal plants used in traditional medicinein Algeria for its potential. The goal of this research is to develop novel bioactive natural compounds by valorizing medicinal and aromatic plants found in Algerian flora. There has never been any information about the lectins of *Eucalyptus globules* herba-*in vitro* antioxidant properties.As a result, we investigated the effect of *Eucalyptus globulus* lectin on LPS-induced kidney injury and the mechanisms underlying, including stress apoptosis and inflammation.

Materialsand methods

Plant material

Aerial parts of *Euclayptus globbulus* were collected during the flowering phase (November2020) from Constantine, which is located in the Northeast of Algeria (The plant material was cleansed, broken up, and tossed around in the air).

Chemicals

Sigma Chemical Company provided all of the chemicals utilized in this project.

Experimental animals

Male albino mice, weighing 160-350 g, are housed in individual stainless steel cages. They were in a space with a temperature of 21±1°C and up to 12 hours of light per day. Six experimental groups of six rats each were formed from the rats.

The animals were fasted for 12 hours before being divided into five groups (each with six animals) as described below:

Group 1: served as the control, treated with normal saline.

Group 2: treated with reference drug diclofénac at a dose of 15mg/kg for 14 days.

Group 3: treated with purified lectin only at a dose of 3mg/kg by intraperitoneal injection for 14 days.

Group 4: treated with lipopolysaccharide at a dose of 200µg/kg by intraperitoneal injection for 14 days.

Group 5: treated with reference drug diclofénac at a dose of 15mg/kg 30 min before lipopolysaccharide injection for 14 days.

Group 6: treated with purified lectin at a dose of 3mg/kg by intraperitoneal injection 30 min before lipopolysaccharide injection for 14 days.

Blood was taken by retro-orbital sinus puncture from each rat 24 hours following the last injection.Rats were decapitated and their kidneys extracted after that.

Tissue preparation

At 4°C homogenates were prepared with 500mg of kidney in 4ml of buffer solution of phosphate-buffered saline; both were homogenized with ultraturax, and centrifuged at 10.000xg for 15min. The reduced glutathione (GSH), as well as superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), and catalase activities were all determined in the supernatant.

Determination of reduced glutathione (GSH)

The level of reduced glutathione (GSH) in the liver was determined spectrophotometric ally using Ellman's reagent (DTNB) as a colouring reagent, as reported by Weeckbekeretcory (1988).

Superoxide dismutase activity (SOD)

The ability of SOD to prevent the photo reduction of nitro-blue tetrazolium (NBT) was measured to determine its activity; the activity of superoxide dismutase (SOD) (EC.1.15.1.1) was measured using an Asada *et al*. technique (1974).

Determination of GSH-Px (E.C.1.11.1.9)

Glutathione peroxidase (EC 1.11.1.9) activity was modified from the method of Flohe and Gunzler (1984)

Catalase activity (CAT)

Catalase (CAT) activity (EC.1.11.1.6) was determined using the Aebi technique (1984).

Protein quantification

Bradford's (1976) method was used to measure protein, with bovine serum albumin as the standard.

Histopathological examination

Autopsied animals' kidneys were removed and fixed in formalin (10 percent).

Using a microtome, five microns thought sections were generated, and these sections were stained with hematoxylin and eosin. These slides were examined under a light microscope for histological changes.

Statistical analysis

For comparison across groups, the data were subjected to an ANOVA one-way test. The data is presented as a mean + standard error of the mean (SEM). $P<0.05$, $P<0.01$, and $P<0.001$ were used as significance levels.

Results

Treatments' effects on body weight, absolute and relative kidney weights

Table 1 shows the effect of lipopolysaccharide, purified of new lectin from Morus nigra, reference drug (diclofenac) and combined treatment with (a new lectin and lipopolysaccharide), (diclofenac and lipopolysaccharide).

A marked increase in rats' body gain was observed in lipopolysaccharide treated rats and a new lectin and diclofenac groups, Along with a new lectin+ lipopolysaccharide and diclofenac + lipopolysaccharide, showed decreased body gain. Lipopolysaccharide-treated rats showed a significantly increased kidney weight and relative kidney weight as compared to control. Combined treatment with purified lectin or diclofenac showed significantly decreased relative kidney weight.

Table 1. Treatments' effects on body weight, absolute and relative kidney weights.

Parameters	Control	Diclofenac	Lectin	LPS	Diclofenac+LPS	Lectin+LPS
Initial body weight(gr)	243.5	264.16	282.2	244.6	281.41	322.4
Final body weight(gr)	238	274.28	331.6	261.85	274.28	243.6
Kidney weight (gr)	1.730	1.523	1.897	1.827	1.919	1.456
RKW (g/100g)	0.726	0.555	0.572	0.697	0.699	0.597

Treatment effects on oxidative stress markers in the kidneys

Lowered glutathione levels, SOD, GSH-Px,GSH and catalase activity, were all not significantly reduced after exposure to lipopolysaccharides. And in lipopolysaccharide-intoxicated rats. Treatment with diclofenac or pure lectin alone did not result in a substantial decrease. There was no significant increase in reduced glutathione level, SOD, GSH-Px, or catalase activities when lipopolysaccharide was coupled with diclofenac or pure lectin.

In comparison to the control, no significant reduction in lipid peroxidation was seen (Figs 1).

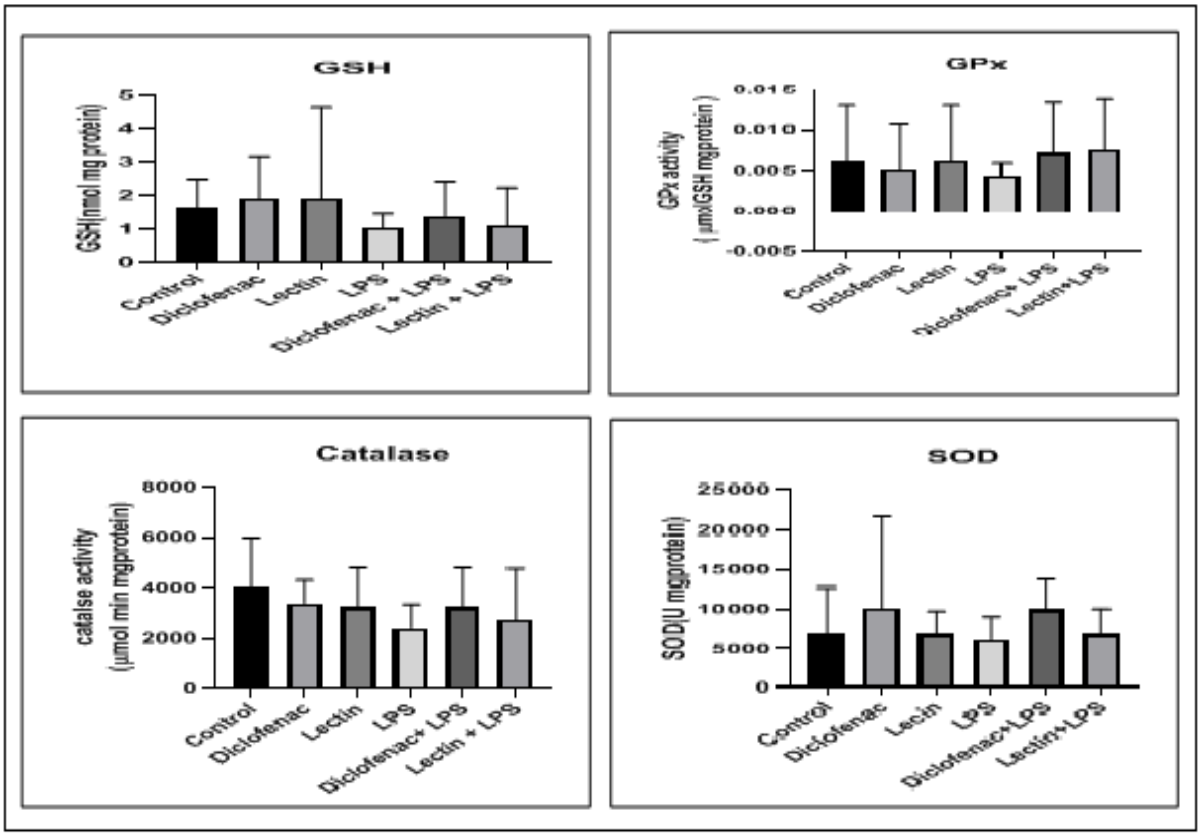

Fig. 1. Glutathione levels and enzyme activities in the kidney of control and rats treatedwith Lectin, Diclofenac, LPS and combined treatment of LPS with Diclofenac or Lectin after 14 days of treatment. Values are given as mean ± SEM for a group of 6 animals, each significant difference: * compared to controls (*P≤0.05; **P≤0.01; ***P≤0.001).

Histological studies

Fig. 2 shows the histological alterations in the kidney.LPS caused a variety of degenerative changes in rats' kidneys. Renal tubular injury, indicating tubular necrosis, was present in these changes.

Lectin or diclofenac administration with LPS resulted in reparative alterations in the combination group. The kidney demonstrated significant improvement in the form of normal renal tubular and tubular necrosis Histologically, the kidneys in the control group had a regular structure (Fig.2 A). Furthermore, there were no histological changes in the kidneys of the lectin or diclofenac-treated groups (Fig.2A).

Discussion

In the current work, LPS-induced oxidative stress was demonstrated in the kidneys of rats by an increase in lipid peroxidation and inhibition of SOD, GSH-Px, and catalase activities. Biological membranes are harmed as a result of lipid peroxidation, resulting in cellular damage.

Tubular damage has a significant role in the lowering of glomerular filtration rate in acute tubular necrosis, according to animal studies. (Necib *et al*, 2013); the loss in glomerular function in LPS-treated rats could be due to two primary tubular abnormalities: Glomerular Filtrate Obstruction and Backleak. Changes in glomerular function in LPS-treated rats could potentially be due to reactive oxygen species (ROS), which cause mesangial cells to contract. Reducing the glomerular filtration rate by changing the filtration surface area and ultrafiltration coefficient variables. The ability to clear free radicals from the organism is represented by the activity of SOD, GSH-Px, and Catalase, which can clear to protect cells against injury. LPS reduced GSH levels, SOD, GSH-Px, and Catalase activities,as well as causing histological damage.

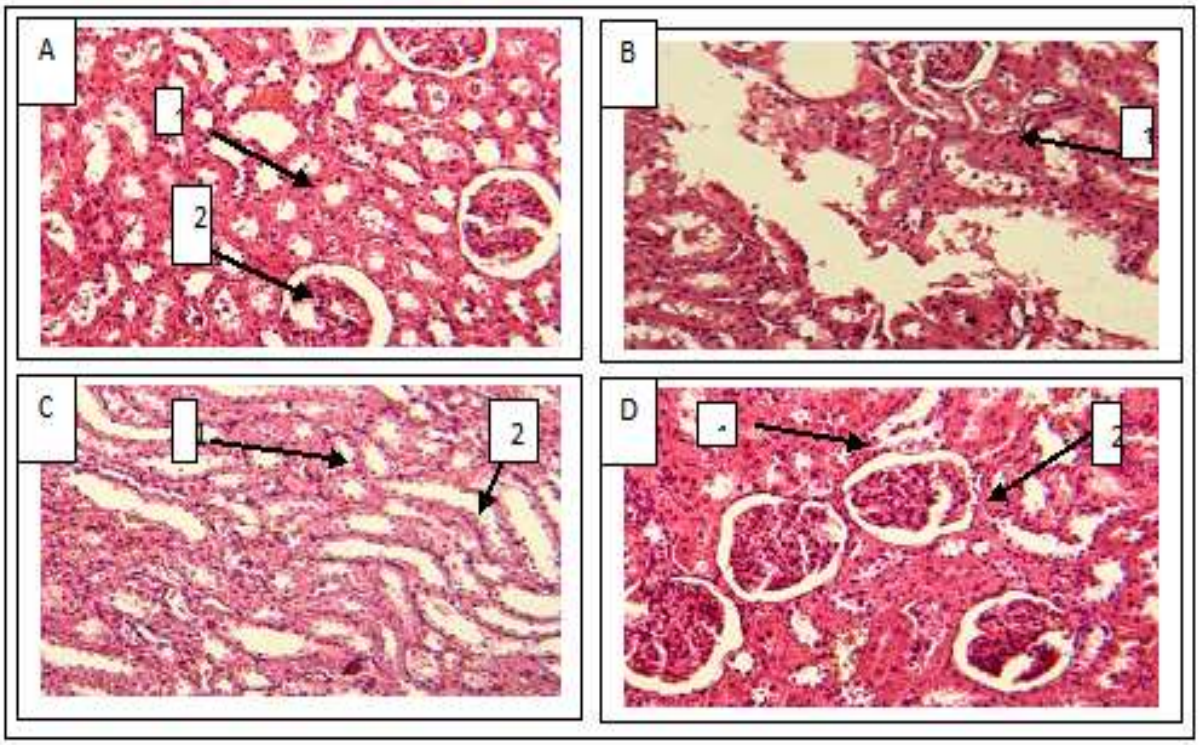

Fig. 2. T.S. of kidney of male rat treated with Lipopolysaccharide (LPS) alone, and in combination with Diclofenac or Lectin. (A) control (H&E400X): showing well develop glomerulus (1), with normal tubular cells; (B) Lectin alone treatment (H&E 400X): showing normal glomerulus(1), and normal tubular cells; (C) LPS treatment (H&E100X): showing degeneration of tubular cells (1), loss of nuclus (2), degeneration of glomerulus (3); (D) combined treatment of LPS with lectin (H&E400X): showing normal glomerulus (1), normal tubular cells (2).

The purpose of this study was to explore at the health impacts of LPS-induced renal oxidative stress and inflammatory responses, as well as the potential protection provided by *Eucalyptus globulus* lectin injection.

LPS-induced kidney damage is known to be caused by oxidative stress, and the resulting redox imbalance may result in the depletion of endogenous antioxidants such as antioxidant enzymes and a change in GSH redox state. As a result, bolstering the antioxidant defense system is required, particularly during infections or periods of chronic oxidative damage. Whole extracts or isolated chemicals from plants are commonly used to reverse and/or prevent kidney toxicity and oxidative stress caused by noxious agents like LPS, and these positive effects are due to their antioxidant and anti-inflammatory capabilities. In this investigation, it was discovered that LPS administration had no significant impact on the rats' body weight gain, absolute kidney weight, or relative kidney weight.

The current investigation found that 30 minutes before the LPS injection, supplementing with the isolated lectin of *Eucalyptus globulus* prevented the caused kidney injury.

The potential of the proteins to stabilize and preserve the integrity of the renal membrane may explain the protective effect of pure *Eucalyptus globulus* lectin demonstrated in our investigation. Such as stimulating renal cell renewal and protein synthesis to heal damaged renal tissues; Lipid peroxidation generated by LPS is a marker of oxidative stress, and multiple prior investigations have found increased lipid peroxidation in a variety of rat organs (including the liver, heart, brain, small intestine, and stomach) (Kaur *et al*, 2006, Sebai *et al*, 2010). Under conditions of oxidative stress, reactive oxygen and

nitrogen species (RONS) attack the polyunsaturated fatty acids (PUFAs) of cell membranes, causing destabilization, disintegration and alteration in membrane fluidity and permeability, all events which increase the rate. This causes protein breakdown and, inevitably, cell lysis (Pari *et al*, 2010). Lipid hydroperoxide chaotic cross-linking with proteins and decomposition products such *as MDA and 4-HNE can also produce* nucleic acids. Resulting in protein oxidation and DNA damage. The potential of lectin isolated from Morus nigra to scavenge free radicals has never been established in vitro, and this has now been confirmed in this study. Because lectin is a protein, it may be able to directly bind RONS and scavenge them, or act as sacrificial antioxidants to stop the lipid peroxidation cascade, as demonstrated in this work. A crucial stage in LPS-induced kidney damage is the weakening of the antioxidant defense mechanism. An LPS attack is characterized by changes in tissue and circulating antioxidant enzyme levels, as well as non-enzymatic antioxidants such as GSH, according to evidence .This suppression is unsurprising because O2• has been implicated as one of the toxic mediators responsible for the majority of toxicities reported in LPS-induced cellular injury, and SOD, a metalloprotein, is a crucial enzyme engaged in this process by spontaneously dismuting O2• to H2O2.The hemoprotein CAT, which is found in the peroxisomes, normally decomposes the H2O2 generated by SOD into water and oxygen. The glutathione defense system includes enzymes like GPx, GST, and catalase. Meanwhile, GPx requires GSH as a co-substrate to catalyze the reduction of H2O2 and lipid hydroperoxides. In this research, LPS treatment significantly reduced the activities of both GPx, and catalase. A sign of their deactivation and the antioxidant enzymes' failure to counteract the influx of RONS caused by LPS exposure. The findings also demonstrated that feeding lectin isolated from *Eucalyptus globulus* for 30 minutes before the LPS treatment for 14 days corrected the alterations in SOD, GPx, and CAT activity in the kidney. The modification of antioxidant enzyme activities seen in LPS-injected rats consuming *Eucalyptus globulus*lectin could be attributed to the direct soaking of RONS produced by LPS because lectin antioxidant activity is well-known as a free radical scavenger. In addition, up-regulation of antioxidant enzyme gene expression could be another mechanism that needs to be investigated further in future studies. In-plant and animal cells, reduced glutathione (GSH) is the most abundant non-protein thiol. It is necessary for a range of biological processes to be regulated. plays a vital function in intracellular defense versus ROS and other free radicals (Kono *et al*, 1982).It can act as a nucleophile, creating conjugates with numerous xenobiotics and/or their metabolites, and also as a reduction agent in the decomposition of hydrogen peroxide and other organic peroxides, thanks to its sulphydryl (–SH) group (Naik *et al*, 2011). The –SH group of GSH is oxidized when it comes into contact with free radicals, resulting in the production of a disulfide molecule (GSSG).During situations of oxidative stress, GSH depletion is frequently accompanied by an increase in GSSG concentration and a reduced GSH:GSSG redox ratio (Suntres, 2011; Dickinson, 2002). GSH levels were found to be lower in rats given LPS injections in the current investigation. GSH levels in both tissues of LPS-challenged rats and LPS-treated rats were given lectin. Decreased always as a result of these occurrences, GSH levels were recovered to those seen in negative control mice, demonstrating that lectin isolated from *Eucalyptus globulus*can prevent LPS-induced glutathione imbalance. The impact of lectin on lipopolysaccharide-induced oxidative stress in rats is corroborated by biochemical and histological data.

Conclusion

The study, the generation of reactive oxygen and nitrogen species was promoted by LPS. The findings of this study also show that lectin isolated from *Eucalyptus globulus* can reduce kidney damage, as well as decrease LPS-induced oxidative stress and inflammatory responses in the kidney. In a Wistar rat model, as well as redox (GSH:GSSG) imbalance. The antioxidant capabilities of lectin isolated from *Eucalyptus globulus* may explain some of the reported anti-inflammatory action. This suggests that lectin isolated from *Eucalyptus globulus* could be useful in the prevention of LPS-induced kidney injury, although more research is needed.Future research is needed to fully understand the mechanisms underlying the protective effects of this purified lectin.

References

Aebi H. 1984. Catalase in vitro. Methods Enzymol **105,** 121–126.

Asada K, Takahashi M, Nagat M. 1974. Assay and inhibitors of spinach superoxide dismutase. Agriculture Biologie Chemical **38,** 471-47.

Bradford MA. 1976. Rapid and sensitive method for the quantities of microgram quantities of protein utilizing the principale of protein-dye binding Anal biochemical **72,** 248-54.

Chen DQ, Hu H H, Wang YN, Feng YL, Cao G, Zhao YY. 2018. Natural products for the prevention and treatment of kidney disease. Phytomedicine **50,** 50–60.

Dickinson DA, Forman HJ. 2002. Glutathione in defense and signaling. Annal of Academic Sciences, **973(1),** 488–504.

Feng. 2015. The Role of Oxidative Stress and Antioxidants in Liver Diseases. International Journal of Molecular sciences **16,** 26087–26124.

Flohe L, Gunzler WA. 1984. Analysis of glutathione peroxidase. Methods enzymol **105,** 114-21.

Kaur G, Tirkey N, Chopra K. 2006. Beneficial effect of hesperidin on lipopolysaccharide-induced hepatotoxicity. Toxicologie **226,** 152–160.

Kono Y, Fridovich I. 1982. Superoxide radical inhibits catalase. Journal of Biologie and Chemical. **257,** 5751–5754.

Morrell ED, Kellum JA, Pastor-Soler NM. 2014. Septic acute kidney injury: molecular mechanisms and the importance of stratification and targeting therapy. Critical Care **18,** 501.

Naik SR, Thakare VN, Patil SR. 2011. Protective effect of curcumin on experimentally induced inflammation, hepatotoxicity and cardiotoxicity in rats: Evidence of its antioxidant property. Experimental Toxicologie and Pathologie **63,** 419–431.

Necib Y, Bahi A, Zerizer S, cherif A, Boulakoud MS, chettoum A, Hallas A. 2013. Effect of argan oil (Argania Spinosa .L) on kidney function impairment and oxidative stress induced by mercuric chloride in rats. International journal of sciences review and research **22(2),** 144-148.

Pari L, Shagirtha K. 2010. Hesperetin protects against oxidative stress related hepatic dysfunction by cadmium in rats. Experimenta lToxicologie and Pathologie **64(5),** 513–520.

Peerapornratana S, Manrique-Caballero CL, Gómez H, Kellum JA. 2019. Acute kidney injury from sepsis: Current concepts, epidemiology, pathophysiology, prevention and treatment. Kidney Int. **96,** 1083–1099.

Rapa Shara F, Biagio R, Di Iorio, Pietro Campiglia, August Heidland, Stefania Marzocco. 2020. "Inflammation and Oxidative Stress in Chronic Kidney Disease—Potential Therapeutic Role of Minerals, Vitamins and Plant-Derived Metabolites" International Journal of Molecular Sciences **21(1),** 263.

Sebai H, Sani M, Yacoubi MT, Aouani E, Ghanem-Boughanmi N, Ben-Attia M. 2010. Resveratrol, a red wine polyphenol, attenuates lipopolysaccharide-induced oxidative stress in rat liver. Ecotoxicologie and Environement Safity **73,** 1078–1083.

Sha Li, Hor-Yue Tan, NingWang, Zhang-Jin Zhang, Lixing Lao, Chi-Woon Wong, Yibin Suntres ZE. 2011. Liposomal antioxidants for protection against oxidant-induced damage. Journal of Toxicologie **(152474),** 16.

Weckbercker G, Cory JG. 1988. Ribonucleotide reductase activity and growth of glutathione-depended mouse leukaemia L 1210 cells in vitro. Cancer letter **40,** 257-264.