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Phytochemical Analysis and Evaluation of Antioxidant Properties of Extracts from Leaves, Stem-Barks and Roots of *Pterocarpus Erinaceus*

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Abstract

Oxidative stress is found to be associated with the etiology of several human diseases. Free radicals are the causative agent of oxidative stress, and can be scavenged by either natural (plants) or synthetic antioxidants. Synthetic antioxidants have some side effects which push a lot of research focuses on natural antioxidants. Pterocarpus erinaceus is a medicinal plant employed as traditional remedy for the treatment of several diseases associated with oxidative stress. This basis, the present study aimed at screening phytochemical and evaluating antioxidant properties of extracts from leaves, stem-barks and roots of Pterocarpus erinaceus. Leaves, stem-barks and roots of Pterocarpus erinaceous after collection were air-dried and pulverized. Each was extracted with methanol and the methanolic extracts were used. Phytochemicals were screened, in vitro, and in vivo antioxidant studies conducted. Rats were grouped into; Group 1: Normal control (liquid paraffin, vehicle 1 ml.kg), Group 2: Negative control (received 1 ml/kg CCl_4), Group 3: Positive control (received 1ml/kg CCl_4 +1 ml/kg Silymarin), Group 4-6: Extract treated rats (received 1 ml/kg CCl_4 + varied doses of Extracts at 100, 200, and 400mg/kg body weight of rats). The treatment was done daily via oral means for 14 days. The results of the study showed Pterocarpus erinaceus rich in different phytochemicals which possess free radicals scavenging properties. In vivo study revealed that Pterocarpus erinaceus could ameliorate CCl_4 toxicity in a way that seem to be via elevation of endogenous antioxidant activities. In conclusion, the leaves of Pterocarpus Erinaceus possessed more of the components rich with antioxidant properties hence its recommendation for further study to identify molecules enshrined therein.

Keywords: *Pterocarpus Erinaceus,* Phytoconstituents, Free radicals, Methanolic extracts, Rats

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INTRODUCTION

In a developing country like Nigeria, use of medicinal plants in the management of diseases associated with oxidative stress is on the increase due to the challenges in high cost of available drugs and some advance side effects. Medicinal plants remain one of the most important remedy due to their availability and affordability as well as their safety (Meenakshi *et al*., 2011) This has drawn the attention of many researchers to focus on medicinal plants for evaluation of antioxidant phytochemicals which have received more attention for their potential role in prevention of human diseases (Upadhyay *et al*., 2010).

The existence of healthy tissue is based on the protection versus oxidative injury induced as a result of excess free radical species (Mugoni *et al*., 2013). The liver is the most crucial organ that exhibits the vital role in safeguarding several physiological processes in the body. It is involved in several imperative functions, as metabolism, excretion, and storage. Liver provides a basic function in the detoxification of endogenous and exogenous intermediaries. Consequently, liver injuries is accompanied by crucial implications for the health of the affected person (Ilyas *et al*., 2016). Liver injuries that are associated with oxidative stress have been a major research focus by many scientific studies (Beretta and Facino, 2010; Niki, 2010). Oxidative stress is developed when there is an excess production of ROS on one side and a deficiency of antioxidant molecules (Giasson *et al*., 2000). Despite the growth in the production of agents with efficacies to reverse the damage induces on the liver, hepatic injuries still remain a global challenge with a serious concern to the health system (Asrani *et al*., 2019). In this regard, exploration of more alternative therapeutic medicine without severe side effects is vigorously required. In line to this, therefore, herbal medicines ought to be re-evaluated as new dynamic therapeutic agents with minimal side effects (Tong *et al*., 2015).

The use of plants as alternative medicine is dated back to centuries, even before long recorded history (Jamshidi-Kia *et al*., 2018). People valued and appreciated the great diversity and importance of plants that are accessible to them (Li and Lou, 2018). As times passed by, so may people and groups have added the medicinal power of herbs in their field to its knowledge base (Dereli *et al*., 2019). Thus, in the exploration of many more reliable and safer liver protective agents, medicinal plants play a significant role (Datta *et al*., 2023). Medicinal plants being an effective source of both traditional and modern medicines are gaining more ground for use in primary health care (Santos *et al*., 1995; Oteng Mintah *et al*., 2019). Many plants and plant products have been recommended for use in the treatment of liver diseases (Govind, 2011).

The plant is a tree found in the most tropical areas of Africa (Tittikpina *et al*., 2018). In West Africa, its leaves, stem bark, and roots have been reported as highly use for traditional remedies against inflammation, ulcer, pain in the joints, malaria-fever, and bacterial infections (Noufou *et al*., 2017).Various scientific studies had confirmed *Pterocarpus erinaceus* ability to exhibit several biological activities as well as identification of several components. For example, analysis of *Pterocarpus erinaceus* aqueous extract has revealed the presence of catechin and epicatechin compounds and had also reported the inhibitory ability of the extract against γ-secretase activity (Hage *et al*., 2015). The bark extract of *Pterocarpus erinaceus* was found to contain friedelin, lupeol, and epicathechin compounds and was able to exert anti-inflammatory, analgesic, and antioxidant activities in a study conducted by Ouedraogo *et al* (2012).

The present study analyzed the phytochemical constituents of the different parts (leaves, stem-barks, roots) of *Pterocarpus erinaceus* and also evaluated their free radical scavenging

potential (*in vitro*) as well as their influence on endogenous antioxidant molecules (*in vivo*). Antioxidant activity has been reported as part of various medicinal properties of *Pterocarpus erinaceus,* however, the plant part that might be rich in this regard has not been ascertained hence this study aimed to identify the part for proper utilization as source for antioxidant components.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used for this study were of analytical grade. Chemicals and solvent were purchased from Sigma Chemical Co. (USA) and Merck (Germany) respectively. Different parameters analyzed in the present study were estimated using commercial kits following manufacturers instructions.

Experimental Animals

Thirty (30) male sistar Strain albino rats weighing between (100-120 g) was used for this study. The rats were purchased from the animal house of University of Jos. The rats were allowed to acclimatize to the environment and were maintained on standard laboratory diet (Vita feed, Jos) and tap water for a period of two weeks. Animals were housed in clean cages under normal prevailing environmental condition. The principles of laboratory animal instituted by National Institute of Health (1985) were followed, as well as specific national laws where applicable. All experiments conducted in this study were monitored by the appropriate ethics committee of the University of Jos, Nigeria.

Plant Collection, Identification and Processing

The leaves stem-barks and roots of *Pterocarpus erinaceous* were collected from Tulu Village of Toro Local Government in Bauchi State. It was then taken to the Plant Science Department of the University of Jos for identification. The leaves, stem-barks and roots of *Pterocarpus erinaceous* were washed and air dried at room temperature. These samples were separately pulverized using laboratory mortar and pestle. The powdered samples were then placed in separate bags and stored in desiccator until required.

Extraction

The powdered leaves, stem-barks and roots of *Pterocarpus erinaceous* (500 g) was separately soaked in 2.5 liters of methanol for 24 hours, after which was filtered using a piece of clean, sterile, white Muslin cloth to remove debris and filtered using Whatman No.1 filter paper. The filtrate was concentrated using a rotatory evaporator and then evaporate to dryness using drying cabinet at 40 ^{0}C as done by Saidu *et al* (2007). The dry crude methanolic extracts was stored in an air-tired plastic containers and stored in a refrigerator at 40 ^{0}C until required.

Phytochemical Analysis of *Pterocarpus erinaceous* Extracts

The extracts obtained from the different parts of *Pterocarpus erinaceous* were subjected to phytochemicals screening to identify presence or absence of certain chemical constituents using method as described by Trease and Evans (1989), and Sofowora (1993)

In Vitro Antioxidant Assessment of *Pterocarpus Erinaceous* Extracts

DPPH scavenging activity was used to assess *in vitro* antioxidant activity of plant extracts as described by Kedare *et al* (2011). Plant extracts (0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL) in 4 mL distilled water was mixed with 1 mL DPPH radical (1 mmol) and were measured

spectrophotometrically at 517 nm. % scavenging activity = Absorbance of control-Absorbance of sample/Absorbance of control×100

In Vivo Antioxidant Study of Plant Extract

Induction of hepatic oxidative damage was done according to the method reported by Guntupalli *et al* (2006). Experimental rats were divided in to six (6) groups of five (5) rats each as shown below. Group 1: Normal control (liquid paraffin, vehicle 1mL/.kg), Group 2: Negative control (1 mL/kg CCl_4), Group 3: Positive control (1 mL/kg CCl_4 +100 mL/kg Silymarin), Group 4-6: Extract treated rats (1 mL/kg CCl_4 + varied doses of Extracts at 100, 200, and 400 mg/kg. The treatment was done daily via oral mean for fourteen (14) days period.

Measurement of Endogenous Antioxidant Molecules/Enzymes

Catalase activity was colorimetrically assayed by the method described by Sinha (1972). The reduced glutathione was estimated by the method of Ellman (1952), while superoxide dismutase activity was analyzed by the method of Kakkar *et al* (1984). Malondialdhyde (MDA) was assayed by the method of Okhawa (1999) method.

Statistical Analysis

All data were expressed as mean ± SEM. Differences among groups at various times of the experiment were subjected to a one-way analysis of variance (ANOVA) followed by Benferonimultiple comparison. Graph pad Instat was used for data analysis and $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Quantitative Analysis of Methanolic Extracts Obtained from *Pterocarpus erinaceus*

The results of the quantitative analysis of methanolic extracts of *Pterocarpus erinaceous* leaves, stem-barks and roots are presented in Table 1. The results obtained show the presence of flavonoids, tannins, alkaloids among other phytochemicals. However, anthraquinones is not found in *Pterocarpus erinaceous*is extracts.

Table 1: Phytochemical (Qualitative) Constituents of Extracts from *Pterocarpus erinaceous*

Phytochemical	Stem-barks	Leaves	Roots
Flavonoids	+	+	+
Tannins	++	+++	++
Saponins	+	+	++
Steroids	+	++	+
Saponin glycosides	++	+	+
Anthraquinones	-	-	-
Cardiac Glycosides	+	+	+
Alkaloids	++	+	+
Volatile Oils	+	++	+
Glycosides	++	++	+++
Balsams	++	+++	++

Key: -= Not Detected, + = trace amount, ++ =moderately present, +++ = highly present

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity of Extracts

The DPPH free radicals scavenging activities of methanolic extracts of leaves, roots and stem-barks of *Pterocarpus erinaceus* are comparable with known standard ascorbic acid is presented in Table 2. Analysis of the scavenging effects of leaves, stem-barks and roots' extract of *Pterocarpus erinaceus* revealed all the samples possessed components with free radical scavenging properties which demonstrated a dose-dependent trend and are significantly (P<0.05) different at with the leaves extract being the most potent having scored 82.73 % activity as compared to 87. 22 % by the standard (L-ascorbic acid) antioxidant substance.

The study therefore determined the concentration of the plant extracts required to scavenge 50% of the DPPH radicals (IC_{50}) in this study and found the concentrations in this order; 0.11 mg/mL (root), 0.10 mg/mL (stem) and 0.09 mg/mL (leaves) respectively while the IC_{50} value of the standard (L-ascorbic acid) was 0.21 mg/mL as presented in Figure 1. The study found IC_{50} of *Pterocarpus erinaceous* leaves extract less compared to that of standard drug.

Table 2: Percentage (%)**2,2-Diphenyl-1-picrylhydrazyl** Scavenging Activity of *Pterocarpus erinaceous* Leaves, Stem-Barks and Roots

Conc. of Compound (mg/mL)	L-Ascorbic Acid (%)	Roots Extract (%)	Stem-Barks Extract (%)	Leaves Extract (%)
0.000	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.200	46.81±0.46^{c}	33.16±0.60^{a}	36.44±0.75 ^{a}	39.38±0.30^{ab}
0.400	62.18±0.18^{d}	36.10±0.75^{a}	44.73±1.73^{b}	53.37±1.30^{c}
0.600	73.06±1.03^{c}	51.64±0.46^{a}	50.60±0.17^{a}	66.67±1.05^{b}
0.800	81.35±0.30^{c}	61.31±0.75^{a}	70.47±1.79^{b}	70.47±1.79^{b}
1.000	87.22±0.75^{d}	67.36±0.30^{a}	76.86±0.96^{b}	82.73±0.62^{c}

The values are expressed as mean ± SEM. Values with different superscript letter(s) are significantly different at P<0.05.

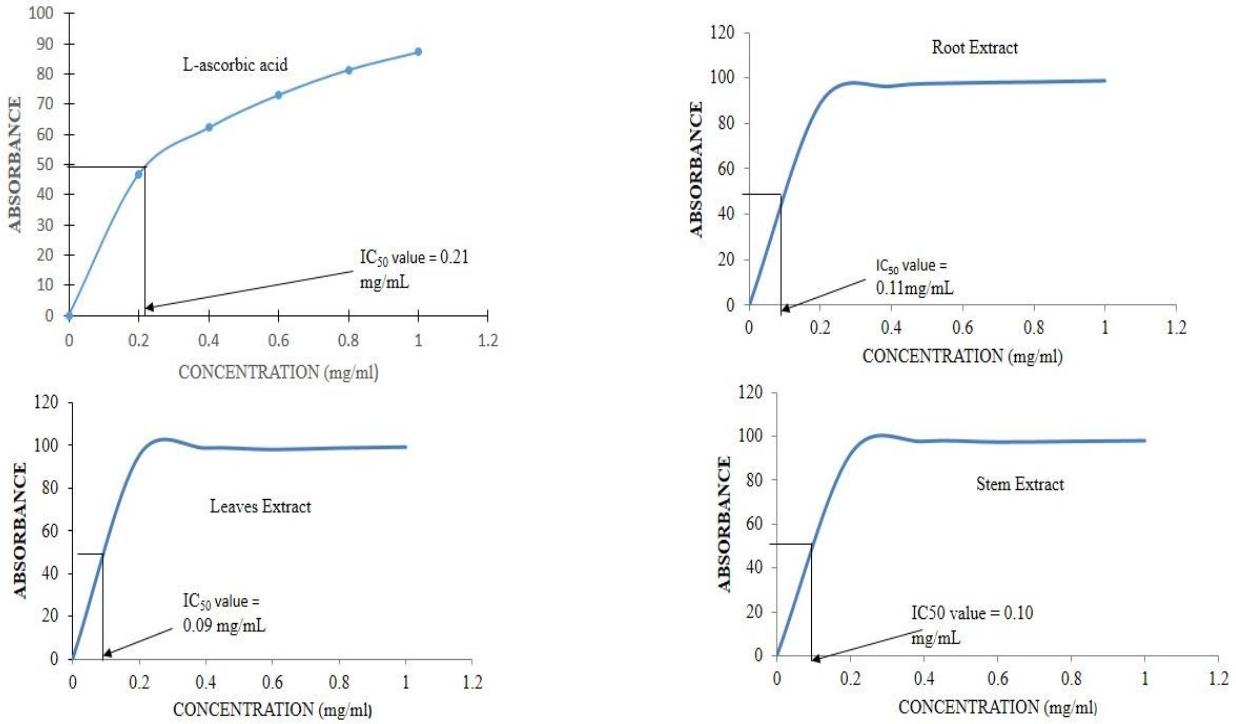

Figure 1. IC_{50} value of Leaves, Stem, and Root Extracts of *Pterocarpus erinaceus* and L-Ascorbic acid against DPPH (**2,2-Diphenyl-1-picrylhydrazyl)**

Antioxidant Activities of Methanolic Extract of *Pterocarpus erinaceus* Leaves

The results of antioxidant activity of the leaves extract of *Pterocarpus erinaceus* on carbon tetrachloride induced hepatic oxidative stress in rats are presented in Table 3. The results show that there was significant increase in malondialdehyde (MDA) in the negative control rats as compared with the normal control and treated rats. Administration of Silymarin caused a significant decrease in MDA as compared with the negative control rats. Administration of methanolic leaves extract of *Pterocarpus erinaceus* caused significant decrease in MDA in a dose increasing manner. The study recorded a significant decrease in catalase (CAT), glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GPX) in the negative control rats when compared with the values from the normal control rats. In the group treated with Silymarin, a significant increase in CAT, GSH, SOD and GPX was recorded. A similar event was observed when varied doses of methanolic leaves extract of *Pterocarpus erinaceus* was administered to different experimental groups where the enzymes activities were found to be elevated significantly ($P<0.05$) with the highest dose of the extract.

Table 3: Antioxidant Activities of Methanolic Extract of *Pterocarpus erinaceus* Leaves on CCl_4-induce Hepatic Oxidative Damage in Rats

Groups/ parameters	MDA (nmol mg^{-1} protein)	CAT (u/mg of tissue)	GSH (mg/100mL)	SOD (u/mg tissue)	GPX (u/mg tissue)
Group 1	3.8 ± 0.12^{a}	29.0 ± 1.58^{a}	222.6 ± 0.93^{a}	0.6 ± 0.05^{a}	184.4 ± 3.07^{a}
Group 2	5.8 ± 0.16^{b}	13.4 ± 1.33^{ab}	129.2 ± 1.88^{b}	0.2 ± 0.03^{b}	84.6 ± 1.50^{b}
Group 3	3.5 ± 0.10^{a}	26.8 ± 1.46^{a}	251.6 ± 1.08^{ab}	0.7 ± 0.04^{c}	167.8 ± 2.18^{ab}
Group 4	5.6 ± 0.13^{b}	20.4 ± 0.93^{b}	186.6 ± 1.66^{c}	0.50 ± 003^{ab}	124.4 ± 1.36^{c}
Group 5	5.3 ± 0.07^{b}	28.8 ± 0.86^{a}	225.8 ± 1.77^{a}	0.50 ± 0.07^{ab}	140.6 ± 1.57^{d}
Group 6	4.3 ± 0.15^{ab}	35.6 ± 1.50^{c}	228.6 ± 2.21^{a}	0.50 ± 0.06^{ab}	175.4 ± 1.86^{ab}

Note: Values are expressed as mean ± SEM of five replicates. Mean values with different superscript letter(s) in a column are significantly different at $P< 0.05$ .Group 1: Normal control (liquid paraffin, vehicle 1 mL/kg) Group 2: Negative control (received 1 mL/kg CCl_4) Group 3: Positive control (received 1 mL/kg CCl_4 +100 mL/kg Silymarin) Group 4-6: Extract treated rats (received 1 mL/kg CCl_4 + varied doses of Extract at 100, 200, and 400 mg/kg body weight of rats). CAT= Catalase, SOD= Superoxide dimustase, MDA= Malondialydehyde, GPX= Gluthionine peroxidase.

DISCUSSION

Medicinal plants are known to possess components with curative potentials of certain biological activity (Oladeji *et al*., 2016, Bhat, 2022). The chemicals which are referred to as active principles or phytochemical substances in plants include flavonoids, terpenes as well as some metabolites such as tannins, saponins, anthraquinones are mostly being the target agent (Mhya and Mankilik, 2014, Angidew, 2022). In an attempt to analyze phytochemicals constituents of the different parts (leaves, stem-barks, and roots) of *Pterocarpus erinaceus,* a screening was conducted on the extracts where some phytochemicals were identified among which include alkaloids, glycosides, flavonoids, cardiac glycosides, steroids and some volatile oil components. These phytochemicals are similar to those identified by Gabriel and Onigbanjo (2010), Sunday (2021) and Okoli *et al* (2023). In order to validate the extracts biological activities, the study carried out an evaluation study by assaying free radical scavenging potential (*in vitro*) as well as their influence on endogenous antioxidant molecules (*in vivo*).

The *in vitro* free radical scavenging study carried out on DPPH revealed that *Pterocarpus erinaceus* extracts possessed ability to scavenge free radical. This is an indication that *Pterocarpus eranaceous* extracts possessed compounds which had free radical scavenging properties. DPPH free radical scavenging has been an accepted mechanism for screening the antioxidant activity of plant extracts (Guchu *et al*., 2020). In an effort to ascertain the most effective plant's part, the study further determined the IC_{50} values of each extract. It has been reported by Abdel-Tawab, ((2021). that, IC_{50} values are indirectly proportional to the antioxidant activity of a compound. The lowest the IC_{50}, the highest the antioxidant capacity and vice versa. By implication, the compound that has an IC_{50} fewer than 50 µg/mL is regarded as a very strong antioxidant, 50-100 µg/mL is a strong antioxidant, 101-150 µg/mL is a medium antioxidant, whereas IC_{50} greater than 150 µg/mL is a weak antioxidant (Wahyuningsih *et al*., 2020; Hussen and Endalew, 2023). In this regard, the leaf, stem-bark and root of *Pterocarpus erinaceus* may be considered to contain molecules with strong antioxidant as evidenced by their IC_{50} values in the range of 0.09-0.11 mg/mL (that is, 90-110 µg/mL) which falls within 50-150 µg/mL of the chart.

Considering the ability of DPPH scavenging and IC_{50} values of *Pterocarpus erinaceus* extracts from the leaves (82.73±0.62% and 0.09 mg/mL), stem (76.86±0.96% and 0.10 mg/mL) and root (67.36±0.30% and 0.11 mg/mL) obtained from this study. By implication, the leaves extract with IC_{50} concentration of 0.09 mg/mL that is equivalent to 90 µg/mL and 82.73±0.62% seems to have possessed components rich with antioxidant activities. Okoli *et al* (2023) have reported a good DPPH scavenging ability of crude ethanol extracts of P. erinaceus leaf. Guided by the *in vitro* study data, leaves extract was selected for the in vivo antioxidant study in order to facilitate the antioxidant potential of *Pterocarpus erinaceus*.

Antioxidant activity has been reported as one of the various medicinal properties of *Pterocarpus erinaceus*. In this study, elevation of MDA in the liver homogenate of the negative control rats is an indicative of oxidative damage by CCl_4. However, extract of *Pterocarpus erinaceus* leaves provided significant protection against the toxicity of CCl_4 on liver of extract treated rats from our previous study (Mohammed *et al*., 2023). To understand the hepatoprotective mystery of the plant leaf, the present study investigated effect of the leaves extract on antioxidant molecules. Carmo *et al* (2022) stated that studying activities of molecules like glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) is key in assessing oxidative stress condition of a tissue because, they are important molecules in both enzymatic and non-enzymatic antioxidant defense system. Increased activities of these antioxidant molecules may suggest their synergetic effect in moping out free radicals (Atchou *et al*., 2021).

Aziz *et al* (2019) reported that, level of reactive species in the cellular system could be reduced by antioxidants either by suppression the expression or activities of free radical-producing enzymes such as xanthine oxidase (XO) and NAD(P)H oxidase, or by promoting the expression and activities of antioxidant enzymes such as SOD, CAT, GSH and GPx. Based on our study, *Pterocarpus erinaceus* leaves seem to exhibit its antioxidant capability via stimulation of the endogenous antioxidant molecules. Both GSH and CAT catalyzes H_2O_2 into $H_2O + O_2$ they by maintaining the integrity of phospholipid bilayer of cells by reducing lipid peroxidation. A decrease in these enzymes leads to a decrease in the endogenous antioxidant system and consequently an increase in lipoperoxidation (MDA). Increase GSH and CAT activities in the extract treated rats in this study is an indicative of extract ability to stimulate the enzymes. Atchou *et al* (2021) have reported elevated activities of endogenous antioxidant enzymes in a study where *Pterocarpus erinaceus* stem-bark extract was used. In the same vein,

GSH activity was also reported to have been activated upon administration of same plant stem-bark extract in rats (Nzute *et al*., 2023).

CONCLUSION

The results obtained in this study have indicated that *Pterocarpus erinaceus*is parts rich in different phytochemicals which possess free radicals scavenging properties. The plant leaves seem to possessed more of the components rich with antioxidant properties. *In vivo* study revealed that *Pterocarpus erinaceus* possessed compounds that had the ability to ameliorate the damage posed by free radicals that have got via elevation of activities of antioxidant molecules. Findings from this study validate the traditional use of *Pterocarpus erinaceus* leaves in the treatment of diseases associated with oxidative stress and is strongly suggested for further research to identify active component(s) that could be developed as an agent to improve treatment of diseases associated with oxidative damage.

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Conflict of Interest

The authors have declared that no conflict of interest exit in regard to this manuscript.

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