Available online at **www.ijmrhs.com**

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2018, 7(5): 136-142

Inhibition of Lipid Peroxidation Induced by γ-Radiation and Dimethylhydrazine in Rat Liver and Brain Mitochondria by Plant Extracts

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ABSTRACT

Contact to dimethyl hydrazine (DMH) or disclosure to gamma radioactivity brings group of responsive oxygen classes (ROC) particularly radical of peroxyl group (ROO•) and radical of hydroxyl group (•OH), which is able to initiate the peroxidation of lipids. The demonstration of early study shows the possibility of extraction therapeutically. Edible plants like Zingiber, Loranthus europeaus, dactylifera, have an important characteristic action called "antioxidant" which is counted as a radical searcher. This study inspected the shielding DMH which lead to cause the peroxidation of lipids by means of brain mitochondria and liver of rats as perfect organisms. The outcomes shows the therapeutic plant excerpts potentially reserved thio-acid and the hydroperoxides of the lipids as active ingredients which demonstrate the effect of the protection of membranes. We conclude that the deep protection consequences due to the outcomes of Zingiber officinale, Citrus aurantifolis, Phoenix dactylifera, Loranthus europeaus in contradiction of the peroxidation of the lipids through two main methods of ROC accomplished of making this kind of harm in a main organelle, the mitochondria as of both rat brain and liver. It also leads to the possibility of using these plants for health benefits.

Keywords: *Zingiber officinale*, *Citrus aurantifolis*, *Phoenix dactylifera*, Mitochondria, Peroxidation, Lipids

INTRODUCTION

Oxidative stress plays an important role in the pathogenesis of various diseases such as atherosclerosis, alcoholic liver cirrhosis and cancer [1]. Oxidative stress can be initiated by an unbalanced production of reactive oxygen species (ROS), such as super-oxide anion (O^{-2}), peroxide radical (HOO•) and hydroxyl radical (•OH). These radicals are formed by one electron reduction of molecular oxygen (O_2). Of pathological significance, ROS can easily initiate a self-propagating lipid peroxidation (LP) of the membrane lipids, which can cause permanent damage to the cell membrane and lipoprotein structure [2]. It is now generally accepted that LP and its products play an important role. Exogenous chemicals and radiation produce peroxidation of lipids leading to structural and functional damage to cellular membranes [3]. Ionizing radiation damages cellular molecules directly by transferring energy or indirectly by generation of oxygen-derived free radicals. Excited states and other reactive species are collectively known as reactive oxygen species (ROS), in liver, kidney and brain toxicity [4-6]. Polyunsaturated fatty acids present in cellular membranes are especially prone to damage by ROS and the resulting LP can have serious consequences. LP plays a major role in mediating oxidative-damage in biological systems. There are also several toxic by-products of peroxidation which can damage other biomolecules away from the site of generation [7,8]. Among the subcellular organelles, mitochondria are one of the key components of the cell killed by radiation-induced oxidative stress. Endogenous antioxidants constitute important defence systems in cells and elicit their action by suppressing the formation of ROS, their scavenging or by repairing the damage caused. Besides this, a number of natural antioxidants are found in plant materials, such as oil seeds, cereal crops, vegetables, fruits, leaves, roots, spices and herbs [9-15]. Some of them exhibit significant antioxidant activity and are commonly utilized for pharmaceutical purposes and in health foods [16-19]. Recent evidence indicates that medicinal plants contain a large number of biologically active components that offer protection against degenerative diseases. A number of medicinal plants have recently been reported to possess significant antioxidant activity [20-22]. Ginger, *Zingiber Officinale* is one of the world's best-known spices, and it has also been universally used throughout history for its health benefits. The dried extract of

ginger contains monoterpenes and sesquiterpenes. The main antioxidant active principles in ginger are the gingerols and shogaols and some related phenolic ketone derivatives. Ginger extract possesses anti-oxidative characteristics, since it can scavenge super-oxide anion and hydroxyl radicals [23,24]. In line with this, gingerol can inhibit ascorbate/ ferrous complex induced lipid peroxidation in rat liver microsomes [25]. Ginger was also suggested to interfere with inflammation processes [26]. Furthermore, ginger acts as a hypolipidemic agent in cholesterol-fed rabbits and can increase the excretion of cholesterol via bile in rats [27-29]. In folk medicine, dates palm *Phoenix dactylifera*, have been used in cases of cold, anemia, asthma, bronchitis, catarrh, cough and congestion, fatigue, fever, flu, diarrhea, hemorrhoids, stomachache, gonorrhea, thirst, toothache, tuberculosis, and vaginitis. In line with this, dates have been reported to exhibit aphrodisiac, contraceptive, demulcent, diuretic, emollient, estrogenic, expectorant, laxative, pectoral, and purgative properties. These beneficial effects can be related to its high content in vitamins and natural fiber, but this fruit can also be an important source of calcium, sulfur, iron, potassium, phosphorous, manganese, copper, and magnesium [30,31]. Key lime *Citrus aurantifolia* is popularly used for treating nausea and locally the juice is a good astringent and is used as a gargle for sore throats [32]. *C. aurantifolia* juice is also a very effective bactericide and can be used for treating rheumatic conditions, malaria and other fevers. The skin of the ripe fruit is carminative [33]. *Loranthus europeaus* has been used in narcotic, antispasmodic, diaphoretic, headache tonic, tearing, rending rheumatic or neuralgic pains, coming on in paroxysms, weak, irregular heart-action with dyspnoea, cardiac hypertrophy, and valvular insufficiency. All parts of the plant contain viscin, also called bird-glue, curiously miscalled birdlime (from the German Vogelleim), deriving its name from the fact that it has been used in Germany in catching small birds. It is very adhesive, soft, and elastic, having a greenish or brownish color, insoluble in water and fixed oils, slightly soluble in alcohol, very soluble in ether. The proof of their antioxidant activity can also explain their mechanism of action and hence, it was considered desirable to evaluate the effect of these plant extracts against lipid peroxidation induced by γ-radiation and 1,2-dimethyhydrazine (DMH), which generates two potent ROS capable of inducing LP, namely hydroxyl radical (•OH) and peroxyl radical (ROO•). Moreover, 1,2-dimethylhydrazine (DMH) generates model peroxyl radicals. These radicals are similar to such peroxyl conditions that are physiologically active [1,2]. The γ-radiation also generates physiologically relevant ROS such as hydroxyl radical, superoxide, hydrogen peroxide, single oxygen, etc. that are capable of damaging many crucial cellular molecules, including membrane lipids [6,12.13]. Hence, inhibition of lipid peroxidation induced by these two agents is physiologically relevant.

MATERIALS AND METHODS

Hydrogen peroxide, ethylene diamine tetra acetic acid (EDTA), 2-thiobarbituric acid, triphenylphosphine (TPP), trichloroacetic acid, xylenol orange and butylated hydroxyl toluene (BHT) were purchased from Sigma Chemical Co, USA, and (DMH) from Aldrich Chemical Co, USA. Other chemicals used in our study were of the highest quality commercially available from local suppliers. The plants selected were *Phoenix dactylifera*, *Loranthus europeaus, Zingiber officinale, Citrus aurantifolia*. The powdered material was defatted with petroleum ether in Soxhlet apparatus for 8-10 hours and the defatted material was then extracted with 70% methanol. Then 0.1 g of the extract was dissolved in 10 ml of distilled water and stirred for 1 hour. Above extracts were centrifuged for 15 min and the supernatants were stored at -20°C. The supernatants were used to examine the antioxidant properties [28]. We had used three different concentrations, i.e. 0.2%, 0.4% and 0.8%. Three-month-old female Wistar rats (weighing about 250 g) were used for the preparation of mitochondria. In brief, rat liver and brain tissues were excised, homogenized in 0.25 M sucrose containing 1 mM EDTA. The homogenate was centrifuged at 3000 g for 10 min to remove cell debris and nuclear fraction. The resultant supernatant was centrifuged at 10,000 g for 10 min to sediment mitochondria. This pellet was washed thrice with 50 mM phosphate buffer, pH 7.4 to remove sucrose. The protein was estimated, and pellets were suspended in the same buffer [3,29]. The mitochondria were suspended in buffer and exposed to γ-radiation from ^{60}Co source at a dose rate of 15 Gy/min. The effect of extract on the oxidative-damage caused by radiation was studied at a dose of 450 Gy. Mitochondria (2.0 mg protein/ml) were suspended in the buffer and exposed to radiation with or without the extracts. The effect of extracts on the oxidative-damage caused by (DMH) was also studied. The mitochondria (2.0 mg protein/ml) were exposed to DMH (10 mM) with or without extract for 30 min. The mitochondria after exposed to γ-radiation and DMH were evaluated for LP. Aliquots (90 ml) of brain/ liver mitochondria, after exposure to radiation sample, were then transferred to microcentrifuge tubes together with 10 ml of TPP in methanol/10 ml of methanol in blank and test samples respectively. The samples were then vortexed and subsequently incubated for 30 min at room temperature. Next 900 ml of Fox II reagent (xylenol orange (100 mM), butylated hydroxy toluene (4.4 mM), sulphuric acid (25 mM), ammonium ferrous sulphate (250 mM)) was added and

samples were incubated for further 30 min in dark. The samples were centrifuged at 12000 g for 10 min prior to reading absorbance of supernatant at 560 nm. The level of peroxide in the sample was then determined using the difference between mean absorbance of samples with and without TPP treatment and the final volume was extrapolated to H_2O_2 concentrations in the standard graph. The effect of plants extracts under study on hydroperoxides induction by DMH at varying time intervals was also determined [31]. Thiobarbituric acid reactive substances (TBARS) assay was performed by standard method using malonaldehyde equivalents derived from tetramethoxypropane. Malonaldehyde and other aldehydes have been identified as products of LP that react with thiobarbituric acid (TBA) to give a pink colored species at 532 nm. The method involved heating of the samples after exposure to radiation and DMH with TBA reagent for 20 min in a boiling water bath. TBA reagent contains 50 ml TCA (20%), 25 ml TBA (500 mg), 2.5 M HCl, 224 mg EDTA and the final volume is made up to 100 ml. After cooling, the solution was centrifuged at 2000 g for 10 min and the precipitate obtained was removed. The absorbance of the supernatant was determined at 532 nm against a blank that contained all the reagents minus the sample. The malonaldehyde equivalents of the sample were calculated using an extinction coefficient of 1.56´ 105 $M^{-1}cm^{-1}$. For collection of endogenous TBARS, fresh samples were boiled without radiation exposure, and values were subtracted [31-32]. Vehicle controls were used for all the extracts. Methanolic extracts, were dissolved in distilled water, for LP experiments.

RESULTS

Data on the effect of plants extracts on lipid hydroperoxides (LOOH) induced by DMH in rat liver mitochondria are presented in Figure 1.

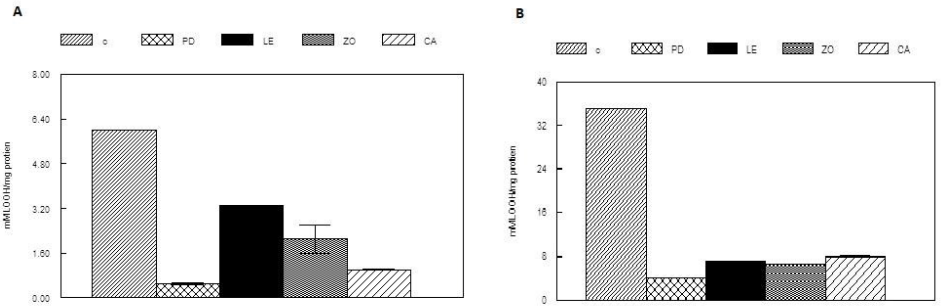

Figure 1 Effect of plant extracts on LOOH formation by DMH in rat liver *Phoenix dactylifera* (PD), *Loranthus europeaus* (LE) *Zingiber officinale* (ZO), *Citrus aurantifolia* (CA). (A) 0 min, (B) 30 min

P. dactylifera with the highest inhibition, was most effective in reducing 1,2-dimethylhydrazine (DMH)-induced LOOH formation, followed by *Zingiber officinale*, *Loranthus europeaus* and *Citrus aurantifolia*. LOOH formation induced by DMH at varying time intervals in brain mitochondria and its inhibition by *Z. officinale* and *Citrus aurantifolia* extracts was more effective than other extracts. LOOH formation was maximum at 60 min after exposure to 1,2-dimethylhydrazine (DMH). After 60 min, LOOH formation was inhibited in all treated groups (Figure 2).

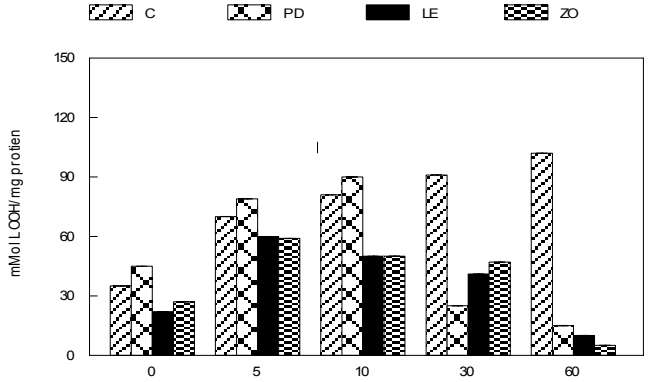

Figure 2 Effect of plant extract *Phoenix dactylifera* (PD), *Loranthus europeaus* (LE) *Zingiber officinale* (ZO), *Citrus aurantifolia* (CA). On LOOH formation induced by (DMH) at various time intervals in rat brain mitochondria

The effect of plants extracts on TBARS formation induced by DMH in rat liver mitochondria is given in Figure 3. *P.dactylifera* and *Citrus aurantifolia* were more effective in reducing LP induced by DMH compared to other plant extracts. Exposure to radiation, as a function of dose, ranging from a dose of 0 to 750 Gy, resulted in enhanced LP as evident by the formation of TBARS. The increase in TBARS formation was significant with the increasing doses examined. Exposure to 300-450 Gy showed steep increases, while higher doses were effective only marginally enhancing peroxidation. Hence the optimum dose of 450 Gy was selected for the experiments, as this dose caused optimum damage in terms of LP in rat liver and brain mitochondria.

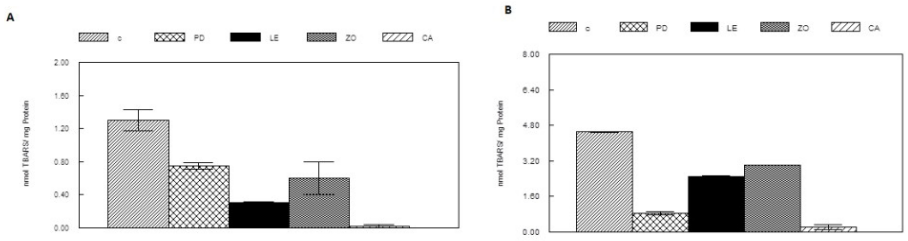

Figure 3 Effect of plant extract *Phoenix dactylifera* (PD), *Loranthus europeaus* (LE) *Zingiber officinale* (ZO), *Citrus aurantifolia* (CA) on TBARS formation induced by DMH in rat liver mitochondria. (A) 0 min, (B) 30 min.

Mitochondria are crucial targets for radiation and free radical-mediated damage. Since mitochondria are devoid of cytosolic antioxidants, as in a whole cell, they are fairly resistant to γ-radiation. Hence a dose of 450 Gy is needed to achieve optimum concentration of free radicals capable of inducing significant damage measurable by simple spectrophotometric means. This dose is much higher than those used in radiotherapy (1-6 Gy) or for radioprotection pertinent to mammals (LD50 in the range of 5-7 Gy).

Such *in vitro* studies with easily measurable systems are usually animal models involving a large number of animals, and being subject to animal ethics committees. Cases in point are such studies on the radio protective effect of caffeine using *in vitro* systems and animal models [33,34]. We plan to extend our investigations to animal studies *in vivo* in future. Data on radiation-induced LP and its protection by plants extracts are given in Figure 4.

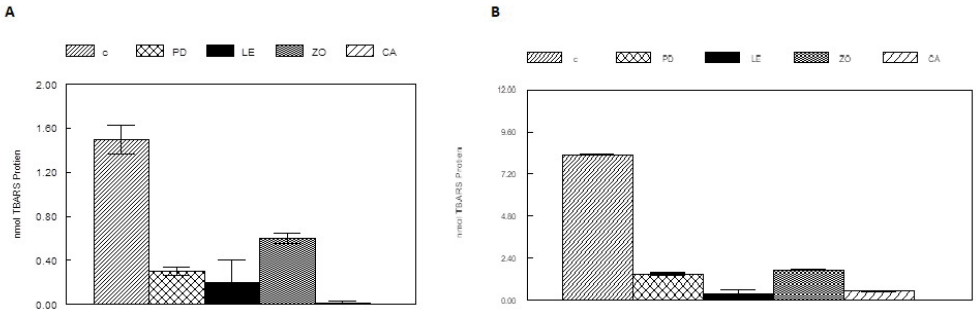

Figure 4 Effect of plant extract *Phoenix dactylifera* (PD), *Loranthus europeaus* (LE) *Zingiber officinale* (ZO), *Citrus aurantifolia* (CA) on TBARS formation induced by γ- ray in rat liver mitochondria. (A) 0 min, (B) 30 min.

Loranthus europeaus and *Citrus aurantifolia* showed significant ability to inhibit radiation-induced LP in rat liver mitochondria. *P. dactylifera* and *Z. officinale* at a concentration of 1% reduced TBARS formation significantly when it was present at the time of irradiation. The formation of LOOH, an intermediate of peroxidation, showed that LOOH formation induced by γ-radiation in rat liver mitochondria was inhibited more effectively by *Z. officinale* and *Citrus aurantifolia* than other extracts, and the data are represented in Figure 5. Prevention of free-radical formation and maintenance of cellular structural integrity and of chemical environment are fundamental requirements of all cells. In biological systems, radiation-induced free radicals impair antioxidant defence leading to increased membrane lipid peroxidation. Generation of ROS by ionizing radiation (especially with low-LET radiation) and DMH, its profound impact on cellular biomolecules are well established [35,36].

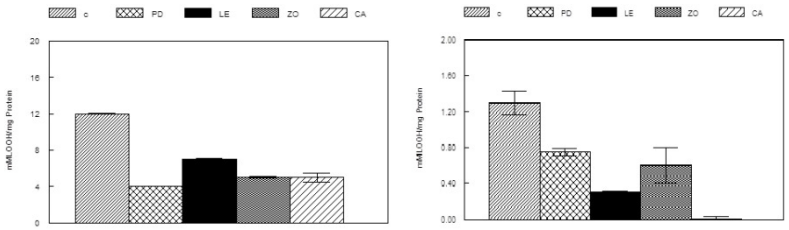

Figure 5 Effect of plant extract *Phoenix dactylifera* (PD), *Loranthus europeaus* (LE) *Zingiber officinale* (ZO), *Citrus aurantifolia* (CA) on LOOH formation induced by γ-ray in rat liver mitochondria. (A) 0 min, (B) 30 min.

The present investigation demonstrates that DMH and radiation induced significant LP in mitochondria. Increase in peroxidation is observed as a function of radiation dose. Radiation generated ROS and is also capable of initiating LP. The initial products of peroxidation are conjugated dienes, to which oxygen is added to form LOOH that further breaks down to stable aldehydes and reacts with TBA to form Thiobarbituric acid-malonaldehyde adduct [37].

Radiation therapy is one of the most important and popular tools for cancer treatment. Because human tissues contain 80% water, the major radiation damage is due to aqueous free radicals, generated by the action of radiation on water. The major free radicals resulting from aqueous radiolysis are $^{\bullet}OH$, $^{\bullet}H$, e^{-}_{aq}, $HO_2^{\bullet}$, H_3O^{+}, etc. [33]. Among them, $^{\bullet}OH$ is the most potent, capable of inflicting severe molecular damage. This free radical reacts with cellular macromolecules such as DNA, proteins, lipids, etc. and causes dysfunction and mortality. These reactions take place in tumor as well as normal cells when exposed to radiation. LP causes membrane damage as well as oxidative modification of critical targets. Agents that can interact with these secondary radicals formed during peroxidation and scavenging them, would be effective in inhibiting LP and in turn protect against radiation and 1,2-dimethylhydrazine induced damage. Removal of excess reactive species, suppression of their generation or protection against peroxidation by repair of membrane damage may be an efficient way of preventing cancer and other diseases.

The effects of plant extracts on LP show significant inhibition of LOOH and TBARS formation. Our earlier studies have indicated that plant extracts are effective scavengers of both primary and secondary radicals [32]. Protection of membranes at both primary and secondary levels explains the possible mechanism by which plants inhibit LP by radiation and 1,2-dimethylhydrazine.

Phenolics are a group of non-essential dietary components that have been associated with inhibition of atherosclerosis and cancer, by chelating metals, inhibiting lipo oxygenases and scavenging of free radicals. Plants phenolic compounds are found to be excellent antioxidants and synergists that are not mutagenic [32]. Our earlier results also have shown that plant extracts possess significant radical scavenging properties of both primary and secondary radicals, in a concentration-dependent manner. Hence the components present in plants may inhibit LP by scavenging of radicals that initiate or propagate LP. Our earlier studies have revealed that all the plant extracts employed in this study possess antioxidant properties, mainly measured as radical scavenging [19,20].

CONCLUSION

The present finding strongly suggests that the use of these extracts to prevent LP leading to membrane damage consequent exposure to radiation and to certain chemicals which generate potent ROS in the form of $^{\bullet}OH$ or $ROO^{\bullet}$. This also explains the possible mechanisms behind the observed health benefits of these plants.

DECLARATIONS

Conflict of Interest

The authors have disclosed no conflict of interest, financial or otherwise.

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