Research Article

Protective role of Maha Vallathy Leghiyam on 4-nitroquinoline-1-oxide-induced experimental oral carcinogenesis

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ABSTRACT

Introduction: The aims of this current study were to examine the effects of Maha Vallathy Leghiyam (MVL) on changes in lipid peroxidation (LPO) status of malondialdehyde (MDA) generation. MDA is a major LPO product that is mutagenic and tumorigenic. The potential protective effect of MVL was 4-nitroquinoline-1-oxide (4NQO)-induced rats. **Materials and Methods:** The male Wistar rats were subjected to the 50 ppm of carcinogen 4NQO through drinking water, and the activity of MVL against the carcinogenic cells was studied through LPO and lipid hydroperoxides (LOOH) hydroxyl radical formation. **Results:** The obtained result was visualized as an increase in the expression of the LPO and hydroxyl radicals in the serum of control and experimental group of animals, these results were associated with the generation of MDA. The administration of MVL (100 mg/kg body weight) protects the generation of LPO products, thereby proves its anticancer potential. **Conclusions:** The administration of MVL has caused significant decrease activities of LPO and LOOH, these aspects suggesting the protective nature of MVL against the carcinogenic nature of water-soluble carcinogen 4NQO by efficiently decreasing the oxidative damage.

KEY WORDS: 4-nitroquinoline-1-oxide, Lipid hydroperoxides, Lipid peroxidation, Maha Vallathy Leghiyam, Malondialdehyde, Rats

INTRODUCTION

Lipids are essential components of the cell membrane and play a key role as signaling molecules in the body. The process of lipid peroxidation (LPO) often observed in the pathogenesis of several diseases and in their clinical conditions, cancer in particular.[1] It was reported that LPO will undergo neuronal degeneration when the free radical flow in the body elevates.[2] In contrast, the product of LPO is malondialdehyde (MDA) and is often used as measure for oxidative stress in the cells.[3] The aldehyde end product is a toxic molecule in nature and its interaction with DNA and proteins associated often leads to potential mutagenesis causing frameshift mutations and base pair mutations in particular and is carcinogenic in rats.[4] The interaction of MDA with deoxyguanosine will result in the production of cyclic adducts. MDA is considered the most convenient biomarker for LPO of the fatty acids' omega-3 and 6, due to their effortless reaction with thiobarbituric acid (TBA).[5] The most major DNA adduct of MDA is pyrimidopurinone of the deoxyguanosine (M1G) and along with this, adenine adducts were also reported to be formed.[6] A very sensitive and analytical method has been developed to quantify these DNA adducts in both human and animal models such as gas chromatography and 32P-post labelling assay.[7] In recent studies, a monoclonal antibody was developed against the M1G adduct, which is coupled to a carrier protein and in addition to, this was reported to have low cross-reactivity with other related endogenous adducts.

Clinical tests are usually carried out by determining the levels of lipid peroxide and the presence of MDA-DNA adducts to determine the pathological condition of the disease.[3] While the elevated levels of lipid peroxidases could be quite harmful and

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fatal, moderate amounts of these could be beneficial for detecting them which, in turn, serves as the second messenger in cell signaling.[8] The reactive oxygen species (ROS) production resulting in LPO is associated with many cancers such as lung cancer, breast cancer, and gastrointestinal.[9] It has been reported that the plasma MDA levels were found to be significantly elevated in patients suffering from lung cancer, breast cancer, colorectal, and prostate cancer.[10-13] In addition to MDA, isoprostanes have also been reported to be in elevated levels in urine of gastric cancer patients. Since MDA has a more prominent occurrence in cancer, it is much more significant after LPO takes place. These findings clearly indicate that in most cancer forms, the LPO end products are elevated and making them major components in the etiology or during the progression of cancers. Mostly, anticancer drugs are introduced to the progressing cancers, while the levels of the lipid peroxidases products will be a major indication to detect the positive action of the drug on the tumor. In this study, Maha Vallathy Leghiyam (MVL) is such anticancer compound whose activity can be detected and evaluated based on its activity against the tumor and the reduction in the LPO end products. To achieve this, a set of experiments is done in this study which includes preclinical animal studies.

MATERIALS AND METHODS

Reagents and Chemicals

4-nitroquinoline-1-oxide (4NQO) was purchased from Sigma-Aldrich Chemicals Pvt. Ltd., Bengaluru, India. All other chemicals used were of analytical grade, purchased from SRL Chemicals Pvt. Ltd., Mumbai, India.

Preparation of MVL

MVL was prepared following the procedure of Siddha Formulary of India (Siddha Formulary of India, Ministry of Health and Family Welfare, Government of India, Part II, 1992) and procured as prepared from Indian Medical Practitioners Co-operative Pharmacy and Stores Ltd., Thiruvanmiyur, Chennai, India. The raw materials used were authenticated before the study. MVL was checked for its solubility in different solvent system and it was confirmed that water was a better solvent to completely dissolve MVL in time-dependent manner. The aqueous extracts of MVL were used for further studies for toxicological assessment.

Experimental Procedure

Male Wistar albino rats (*Rattus norvegicus*) weighing 120–140 g were used for evaluating the chemopreventive nature of MVL. The rats were obtained from Sathyabama Institute of Science and Technology, Centre for Laboratory Animal Technology and Research, Chennai, India. The animals were acclimatized to laboratory conditions for 7 days before the experiments. The rats were maintained at a room temperature of 22–24°C, with a 12 h light/dark cycle and humidity around 50 ± 5%. During acclimatization, the rats were randomized into experimental and control groups and housed individually in sanitized polypropylene cages housed with sterile paddy husk as bedding. Animals were given free access to standard pellet diet and water *ad libitum*. All experimental procedures were in compliance with the Animal Ethical Committee, Committee for the Purpose of Control and Supervision of Experiments on Animals and were approved by Sathyabama Ethical Committee with an approval number SU/CLATR/IAEC/X/082/2018.

Experimental Design

The experimental animals were divided into four groups, each group comprising six animals.

- Group I: Control animals treated with corn oil thrice a week orally for 20 weeks
- Group II: Oral carcinoma was induced by administration of 50 ppm 4NQO dissolved in drinking water for 20 weeks
- Group III: Animals were treated with MVL (100 mg/kg body weight) dissolved in water thrice in a week orally. MVL treatment was started 1 week before the first dose of 50 ppm 4NQO administration (as in Group II) and continued till the end of the experimental period
- Group IV: Animals were treated with MVL (100 mg/kg body weight) dissolved in water thrice in a week orally for 20 weeks to assess the cytotoxicity if any, induced by MVL, and rats were referred as drug control.

After the experimental period, the rats were fasted overnight and anesthetized using diethyl ether and sacrificed by cervical decapitation. A portion of tongue was used for homogenized in 0.1 M Tris-HCl buffer pH – 7.4 and centrifuged. The supernatant was used for biochemical studies, and total protein in serum and tissue homogenate was done.[14]

Assay of LPO

The level of lipid peroxides in serum was assayed by the method of Ohkawa *et al.*[15] To 0.2 ml of plasma/tissue homogenate, 0.2 ml of sodium dodecyl sulfate, 1.5 ml of acetic acid, and 1.5 ml of TBA were added. The mixture was made up to 4 ml with water and then heated in an oil bath 95°C for 60 min using glass ball as a condenser. After cooling, 1 ml of water and 5 ml of n-butanol/pyridine mixture were added and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance at 532 nm was measured.

Lipid Hydroperoxides (LOOH)

The levels of LOOH in serum of experimental rats were assayed by Fraga *et al*., 1988.[16,17] About 0.9 ml of Fox reagent was mixed with 0.1 ml of methanol extracted tissue lipid sample and incubated for 30 min at room temperature. The absorbance was read in a colorimeter at 560 nm.

Statistical Analysis

All the grouped data were evaluated with SPSS/10 software. Hypothesis testing methods included one-way analysis of variance followed by Duncan's multiple range test. $P < 0.05$ was considered to indicate statistical significance. All these results were expressed as mean ± SD for six animals in each group.

RESULTS

Effect of MVL on LPO and LOOH

Figures 1 and 2 show the effect of MVL on the serum level of LPO in terms of MDA and serum level of LOOH in control and experimental group of animals. In 4NQO-induced Group II animals, there was a significant ($P < 0.05$) increase in the level of MDA and LOOH when compared with normal control Group I animals. Whereas in MVL-treated Group III animals, there was a significant ($P < 0.05$) decrease in the level of MDA and LOOH when compared with tumor-bearing (Group II) animals. However, MVL alone treated animals (Group IV) fail to show any significant changes when compared with Group I control animals.

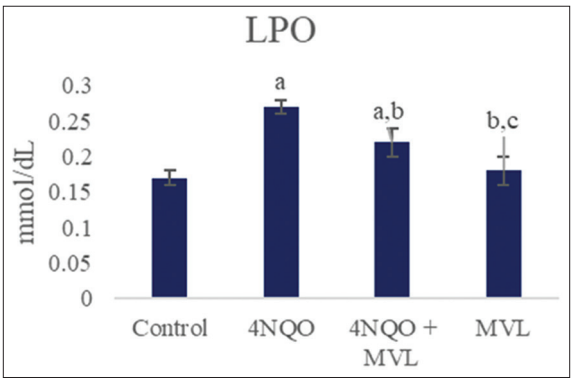

Figure 1: The level of serum lipid peroxidation of control and experimental rats. Results are expressed as mean ± SD for six rats in each group. Statistical significance at $P < 0.05$ compared with [a]Group I, [a,b]Group II, and [b,c]Group II based on Duncan's multiple range tests

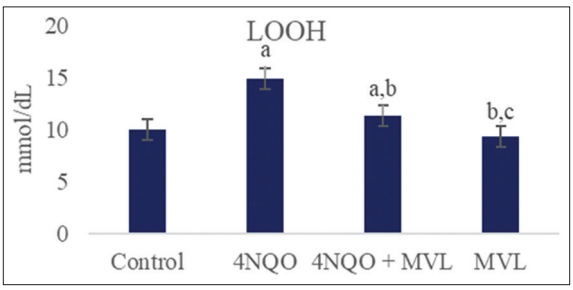

Figure 2: The level of serum LOOH of control and experimental rats. Results are expressed as mean ± SD for six rats in each group. Statistical significance at $P < 0.05$ compared with [a]Group I, [a,b]Group II, and [b,c]Group II based on Duncan's multiple range tests

DISCUSSION

The ROS are the major components responsible for altering macromolecules termed to be oxidative stress. These ROS are usually generated as by-products of cellular metabolism that primarily takes place in the mitochondria. According to many previous studies, it has become evident that the levels of these ROS are much more elevated in cancer cells than in normal cells.[18] The previous studies have reported that increase of oxidative stress is in majority of the cancer types, but very few studies have been done on the evaluation of concentrations of the LPO products in cancer cells.[19] In this study, the experimental animal groups have been evaluated for their levels of serum lipid peroxides. When treated with MVL, the cancer-induced Group II has showed an exceptional increase in the lipid peroxides product (MDA) and hydroxides when compared with the control and Group I animals. This concept was reported by Welsch, in 1995, where he demonstrated the role of lipid peroxides in experimental mammary gland tumorigenesis.[20] The lipid peroxides of the tongue have increased significantly in the cancer-induced set of animals, i.e., Group II when compared with the control Group I. The same repeats in the serum lipid peroxides (MDA) where the induced + MVL-treated Group III rats have shown decreased levels, while Group IV, which is only MVL treated showed no variation and could be correlated to control Group I. Similar testing has been done by Almeida *et al*. in their study of evaluation of oxidative stress in experimental rats.[21] The LOOH levels of the tongue tissue displayed similar results where the induced Group II had significantly elevated levels and Group III (induced + MVL) has displayed decreased levels of LOOH. These were interpreted with respect to the control and Group IV, being only MVL treated showed no change and resembled to the control Group I.

The MVL being very effective against the tumor-induced Group II and showed significant change compared to Group III animals which were treated with MVL. Similar studies have been reported by Sampey BP and executed analysis hepatic MDA during early stages of ethanol-induced liver injury.[22] The oral cancer-induced Group II animals have shown high concentrations of the MDA when compared with the control Group I. Once they were treated with MVL in Group III, they started to recover and regain

the original state indicating the efficiency of MVL and hence showed decreased levels of MDA-DNA adducts. The group MVL alone treated Group IV, however, has shown no change and resembled to that of control Group I animals. Knutson, in 2007, has reported a study which involved the analysis of measurement of the M1G DNA base adduct.[23]

CONCLUSIONS

This work revealed on a biochemical and molecular level that MVL minimized the rat tongue carcinogenesis induced by 4NQO. MVL decreased the serum LPO and LOOH levels. MVL able to inhibit the MDA-DNA adduct formation with the support of various biochemical modulations was reported from our laboratory previously.[24,25] In addition, it induces the apoptosis on the gene and protein levels in *in vitro* conditions. The protective effect of MVL against 4NQO-induced oral carcinogenesis could be explained by its antioxidant properties, this supports our present report on decreased expression of LPO, LOOH, and MDA adduct formation.

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