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# EVALUATION OF *IN VITRO* LIPID PEROXIDATION INHIBITION ACTIVITY OF HEPATOPROTECTIVE POLYHERBAL FORMULATION

**Jayachandra Kuncha[1*], Thirugnanasambantham P.[2], Kumaran S.[3], Narayanan N.[4] and Sharmila Devi V.[5]**

[1*]Research Scholar, Periyar Maniammai Institute of Science and Technology, Thanjavur - 613 403, Tamil Nadu, India
[2]Head, Rumi Herbals R&D Center, Chennai - 600095, Tamil Nadu, India.
[3]Associate Professor, Periyar Maniammai Institute of Science and Technology, Thanjavur - 613 403, Tamil Nadu, India
[4]HOD, Dept. of Pharmaceutics, A.J college of Pharmacy, Chennai - 600119, Tamil Nadu, India.
[5]Senior Research Fellow, Captain Srinivasa Murthy Regional Ayurveda Drug Development Institute, Chennai - 600106, Tamil Nadu, India.

***Corresponding Author: Jayachandra Kuncha**

Research Scholar, Periyar Maniammai Institute of Science and Technology, Thanjavur - 613 403, Tamil Nadu.

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## ABSTRACT

The free radicals are the agents which causes cell membrane damage which leads to formation of lipid hydroxyl and peroxy radicals. This is the primary process in cellular damage. The lipid peroxidation process can preventing through supplying scavengers of free radicals. This is the ultimate reason for supplying antioxidants. Botanicals are the wide source of antioxidants available in nature. The main objective of this study is to evaluate anti-lipid peroxidation activity of the hepatoprotective polyherbal formulation (LVR05) by *in vitro* method. The results shows effective anti-lipid peroxidation activity in dose dependent manner. The maximum lipid peroxidation inhibition was 80.89% at the concentration of 500 µg/ml, at the same concentration standard ascorbic acid shows 91.75% of inhibition. The IC50 values of sample and standard was found 112 µg/ml and 65.74 µg/ml. Based on the results it has been concluded that the polyherbal formulation is effective in prevention of lipid peroxidation. Further work is in progress towards identification and quantification of phytochemicals present in the formulation.

**KEYWORDS:** *In vitro* Lipid peroxidation inhibition, Polyherbal formulation, Free radicals, Phytochemicals.

## INTRODUCTION

Lipid peroxidation is chain process which start with initiation in which polyunsaturated fatty acids are undergo oxidation to form highly unstable components of free radicals. The lipid peroxidation process initiators are reactive oxygen species (ROS) such as hydroxyl (OH•) and peroxyl radicals (ROO•) and the superoxide anion radicals (O2•-), which are formed by exogenous chemicals factors and endogenous metabolic processes in the human body or in food systems[1, 2]. They are linked to many pathological processes which lead to inflammation, cancer, degenerative disease, and other deadly diseases[3, 4].

Antioxidants are the agents which are capable of neutralizing free radicals, ultimately controlling their formation[5]. Prevention of plasma membrane damage is the primary concern in free radical induced oxidative stress condition. In the present study, an attempt has been made to prove the anti-lipid peroxidation efficacy of polyherbal formulation which is formulated by combining five standardized medicinal plant extracts in different ratio. The formulation was coded as LVR05. The individual ingredients present in the formulation was well established for its safety and efficacy. Combining the ingredients may give better effect than individual. Keeping the thought in mind lipid peroxidation inhibition assay was performed by *in vitro* method.

## MATERIALS AND METHODS

### Chemicals and reagents

All the required chemicals and reagents are analytical grade and procured from Loba Chemie Laboratory Reagents.

### Preparation of Polyherbal formulation

Phytochemical extraction was done by cold maceration method by using double distilled water and ethanol in the ratio of 7:3 and kept 48 hours by occasional shaking. The residue was filtered through double lined muslin cloth and evaporated to dryness under reduced pressure by using vacuum evaporator. The resulting greenish black material (LVR05) was kept in airtight container at 4$^{o}$C till it used for experiments.

### Evaluation of *in vitro* Lipid Peroxidation Inhibition assay

The egg yolk was used as lipid source in this study. *In vitro* TBARS assay (Thiobarbituric acid reactive substances) was adopted for evaluation of lipid

peroxidation inhibition with minor modifications[6]. The reaction mixture contains 0.5 ml of (10%) egg yolk homogenate and 0.1 ml of different concentrations of polyherbal formulation dissolved in HPLC grade distilled water and standard ascorbic acid was added to the test tube and made up to 1 ml with distilled water. Add 0.05 ml of 0.07M of FeSO4 to induce lipid peroxidation and incubated at 37 °C for 30 min. Then 1.5 ml of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 ml of 0.8% (w/v) TBA in 1.1% sodium dodecyl sulphate and 1ml of 20% TCA was added and mixed vigorously and then heated at 95°C for 60 min. Allow the test tubes to cool at room temperature and then add 5 ml of butanol, centrifuged at 3000 rpm for 10 min. The supernatant solution was separated and measure the absorbance at 532 nm. The Percentage of inhibition of lipid peroxide formed was calculated as follows:

$$\frac{C-T}{C} X\ 100$$

Where, C - The absorbance of Control, T- Absorbance of Sample or Standard

**Statistical Analysis**

Statistical analysis was performed using GraphPad Prism 5.03 software program. Non linear regression (Curve fit) were performed to find the $IC_{50}$ values.

## RESULTS AND DISCUSSION

**Determination of Anti-Lipid peroxidation activity**

The process of lipid peroxidation is chain process in which biological membrane get damaged. Once damage occurred all the intra cellular organelles will exposed to free radicals. Depending upon the intra cellular component damage the diseases can progress. For example, due to oxidative stress mitochondria may get damaged so the physiological functions of mitochondria like ATP synthesis via oxidative phosphorylation, Tri Carboxylic acid cycle and β-oxidation will get affected. Due to free radicals rough and smooth endoplamicreticulum get damage so protein and phospholipids synthesis get reduced. Lysosomal damage leads to triggering of pro-inflammatory markers. Nucleolus contain genetic materials also affected which leads to mutations and finally oncogenesis.[7,8,9]

**Table 1: Percentage of Lipid Peroxidation Inhibition of LVR05 and Standard in Different Concentrations.**

| S. No | Concentration (µg/ml) | % of Lipid Peroxidation inhibition by Sample | % of Lipid Peroxidation inhibition by Standard |
|---|---|---|---|
| 1 | 7.81 | 7.54 ± 0.69 | 10.42 ± 0.85 |
| 2 | 15.62 | 14.5 ± 1.48 | 17.83 ± 1.57 |
| 3 | 31.25 | 20.29 ± 1.77 | 31.62 ± 3.15 |
| 4 | 62.5 | 33.64 ± 3.47 | 49.12 ± 2.29 |
| 5 | 125 | 54.96 ± 3.95 | 66.23 ± 2.80 |
| 6 | 250 | 69.26 ± 2.24 | 77.73 ± 4.25 |
| 7 | 500 | 80.89 ± 2.10 | 91.75 ± 4.36 |

Values are expressed in Mean ± S.D, (n = 3); Sample - LVR05, Standard - Ascorbic acid

Naturally, there should be balance between the free radicals produced in the body and antioxidants that protect the body against harmful effects (Aline Augusti Boligon *et al.*,2014) Botanical drugs are served as potential source of antioxidants due to presence of diversified phytochemical constituents. In the present study, an attempt has been made to prove the anti-Lipid peroxidation activity of selected polyherbal formulation.

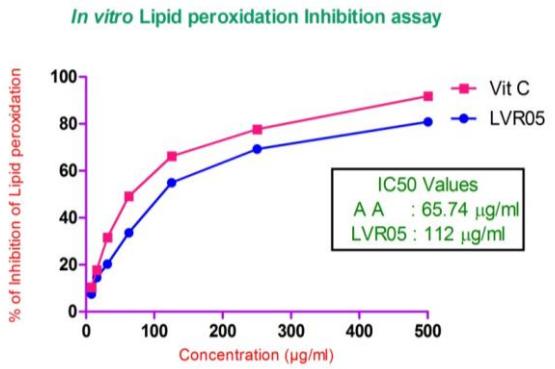


**Fig. 1: Shows dose dependant lipid peroxidation inhibition activity and IC50 values of LVR05 and Standard (A.A: Ascorbic acid)**

The results indicates the polyherbal formulation possess potent lipid peroxidation inhibition activity in dose pendent manner. The highest percentage of lipid peroxidation inhibition was achieved by LVR05 is 80.89 ± 2.10 % at the concentration of 500 µg/ml. At the same concentration standard ascorbic acid shows 91.75 ± 4.36 % of peroxidation inhibition effect (table. 1). The IC50 values were found 112 µg/ml for polyherbal formulation (LVR05) and 65.74 µg/ml for standard ascorbic acid (fig. 1). The phytoconstituents which are present in the formulation are responsible for the activity. Based on above results it has been concluded that, the selected polyherbal formulation (LVR05) possess potent anti-lipid peroxidation activity in dose dependant manner. Hence, the formulation can be used as a therapeutic agent for several disease related lipid peroxidation. Further work is in progress towards identification and quantification of phytochemicals present in the formulation.

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## CONFLICTS OF INTEREST

There are no conflicts of interests.

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