

## Spontaneous Lipid Peroxidation Inhibitory Activity of Standardized Extract of *Ficus racemosa* Stem Bark - An *In Vivo* Study

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### Abstract

**Aim:** To evaluate the antioxidant activity of sequential acetone extract of *Ficus racemosa* Linn. (Moraceae) stem bark in terms of spontaneous lipid peroxidation inhibition in albino rats.

**Materials and Methods:** The *in vivo* antioxidant capacity of sequential acetone extract of *F. racemosa* stem bark was evaluated in plasma of animals receiving a daily dose of 500 mg kg<sup>-1</sup> for 2 weeks. Plasma antioxidant capacity was measured in brain homogenate incubated for 1h at 37°C. The production of oxidized compounds was measured in terms of thiobarbituric acid reactant substances (TBARS) in control and experimental groups. Glutathione was also measured as a marker of oxidative stress.

**Results:** Two weeks of treatment with the extract (500 mg kg<sup>-1</sup>) increased the plasma antioxidant capacity to 93% against 69% in the control group. Glutathione (GSH) depletion was also measured and it was observed that the plasma from extract-treated group showed 81% GSH depletion inhibitory activity, which was significantly higher ( $p \leq 0.05$ ) than that of control plasma (52%).

**Conclusions:** *Ficus racemosa* stem bark extract possesses strong antioxidant activity in terms of lipid peroxidation inhibition and glutathione restoration.

**Keywords:** Antioxidant; Cluster Fig; Glutathione; Lipid Peroxidation; TBARS

### Introduction

Oxidative stress is implicated in several diseases including cardiovascular diseases, ischemic reperfusion injury, cancer, hypertension and diabetes [1]. Free radicals arising from either the normal metabolism or induced by environmental sources interact continuously in the biological systems. Antioxidants are essential for preventing oxidative deterioration of biomolecules and a series of antioxidant compounds are present in the cells, they react with oxidizing agents and disarm them [2]. Recently, attention has been focused on the relationship between reactive oxygen species (ROS) and several disorders including diabetes.

*Ficus racemosa* Linn. (Moraceae) commonly known as 'cluster fig' and found throughout greater part of India in moist locality, is widely used in Indian folk medicine for the treatment of various diseases/disorders including jaundice, dysentery, diabetes, diarrhea and inflammatory conditions [3]. Reports indicate that *F. racemosa* possesses various biological effects such as hepatoprotective, chemopreventive, antidiabetic, antitussive and anti-diarrhoeal [4-8]. We reported *F. racemosa* stem bark to be an excellent source of phenolic compounds and flavonoids exhibiting significant antioxidant activity in terms of radical scavenging, reducing power and anti-lipid peroxidative activ-

ity *in vitro* and *ex vivo* [9]. Since, the antioxidant responses are known to vary *in vivo*, studies involving experimental animals are necessary to measure the antioxidant potential of any extract/component. Further, majority of animal studies involves the use of oxidative stress inducers such as CCL<sub>4</sub>, doxorubicin etc., which evaluate the antioxidant activity of the substance in states of elevated levels of oxidative stress and may be less relevant to the normal biological conditions. In view of the above, the present study evaluated the spontaneous lipid peroxidation inhibitory activity potential of sequential acetone extract in albino rats in the absence of any oxidative stress inducer.

## Materials and Methods

### Plant material and chemicals

*Ficus racemosa* stem bark was collected from Mukkadahally, Chamarajanagar district of Karnataka, India during September 2007, subsequently identified by Dr. Shivprasad Hudeda and the voucher specimen (BOT-001/2008) was deposited at the herbarium of Department of Studies in Botany, University of Mysore, Mysore, India. The bark was cut into small pieces, dried (50°C) and powdered, passed through 60 mesh sieve (BS) and stored in an air tight container at 4°C till further use. All the chemicals and reagents used in the study were of extra pure analytical grade.

### Preparation of the extract

The bark powder was extracted sequentially with solvents of increasing polarity (petroleum ether - chloroform - acetone - methanol - water) in a soxhlet apparatus for 8h each. On evaporation of respective solvents in a rotary vacuum, yielded petroleum ether extract (FR-SPE) - a sticky yellow mass (2.5% w/w), chloroform extract (FRSCE) - greenish sticky mass (1.4% w/w), acetone extract (FRSACE) - red powder (1.5% w/w), methanol extract (FRSME) - a reddish brown solid mass (5.3% w/w) and aqueous extract (FRSAE) - brown powder (4.9% w/w). Acetone extract was selected for the study, because it contained highest amount of phenolic compounds [9]. The extract was standardized using HPLC and was found to contain bergenin and bergapten [10].

### Spontaneous lipid peroxidation inhibitory activity

The *in vivo* antioxidant activity of FRSACE was studied using healthy male Wistar rats weighing 140 - 160g divided into the following two groups each consisting of 10 rats. Schematic representation of the experimental design is shown in figure 1.

- Group I: served as control, received 1 mL normal saline by gavage.
- Group II: served as experimental, received sequential acetone extract (500 mg kg<sup>-1</sup>) dissolved in 1 mL normal saline by gavage.

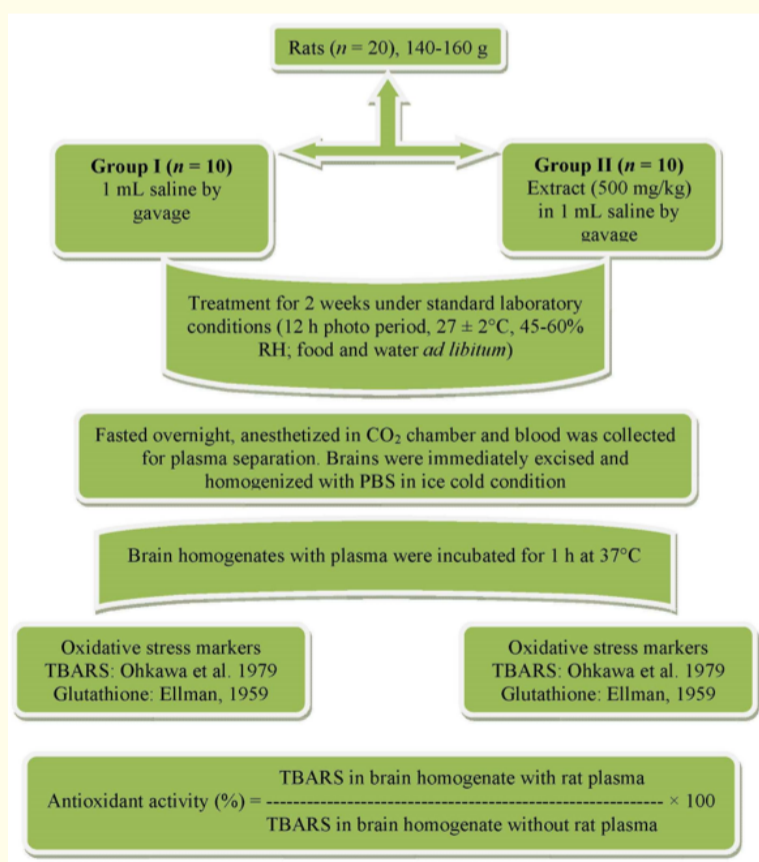


Figure 1: Schematic diagram of the experimental design.

All animal procedures have been approved by the Animal Ethical Committee and performed in accordance with the animal experimentation and care. The rats were housed in polyacrylic cages and maintained at 27 ± 2°C, 45 - 60% RH and 12h photo period and provided with food and water *ad libitum*. Rats maintained with the above treatment for 2 weeks were fasted overnight to minimise metabolic variations, anesthetized in CO<sub>2</sub> chamber and blood was collected by direct cardiac puncture into heparinized tubes for plasma separation. Antioxidative activity was determined as reduction in spontaneous lipid peroxidation of brain homogenate by the addition of plasma [11]. Brain homogenates with plasma were incubated for 1h at 37°C. The contents of glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) as markers of lipid peroxidation were determined by the methods of Ellman [12] and Ohkawa, *et al.* [13] respectively. The results are reported as percent inhibition of oxidation of brain homogenate, and nmoles/μmoles of TBARS and GSH mg<sup>-1</sup> protein for plasma.

The antioxidant activity is the inhibition of TBARS produced in brain homogenate (prepared from adult rats without any treatment) by plasma of the experimental or control group which was calculated using the following formula:

$$\text{Antioxidant activity (\%)} = \frac{\text{TBARS in brain homogenate with rat plasma}}{\text{TBARS in brain homogenate without rat plasma}} \times 100$$

### Statistical analysis

Data were presented as mean ± SD of triplicate determinations. Data were analysed by Student's t test using SPSS 19.0 Software. Values are considered significant at *p* ≤ 0.05.

### Results and Discussion

The present study reports the antioxidant activity of sequential acetone extract of *F. racemosa* stem bark in terms of spontaneous lipid peroxidation inhibition in albino rats. The plasma of FRSACE-treated and control animals were used to evaluate the antioxidant ability of the extract. The TBARS data obtained with brain homogenate revealed less intense plasma peroxidation in FRSACE-treated rats than in the control group. After 2 weeks of treatment, plasma antioxidant capacity was 93% and 69% for FRSACE-treated and the control groups respectively (Table 1). The GSH depletion was also measured and it was observed that the plasma from FRSACE-treated group showed 81% GSH depletion inhibitory activity, which was significantly higher (*p* ≤ 0.05) than that of control plasma (52%).

	TBARS (n moles mg <sup>-1</sup> P)	GSH (μ moles mg <sup>-1</sup> P)
Group I	15.5 <sup>b</sup> ± 0.83	20.4 <sup>a</sup> ± 1.01
Group II	11.3 <sup>a</sup> ± 0.71	33.1 <sup>b</sup> ± 1.19

**Table 1:** *In vivo* antioxidative activity of *Ficus racemosa* bark extract.

\*Group I: control, Group II: sequential acetone extract-treated rats, TBARS: thiobarbituric acid reactive substances, GSH: glutathione, μ moles mg<sup>-1</sup> P: μ moles mg<sup>-1</sup> protein.

<sup>§</sup>Mean values with superscript letters a and b in columns differ significantly (*p* ≤ 0.05).

These observations are in good agreement and further substantiate our *in vitro* and *ex vivo* findings, wherein, various polar extracts (acetone, methanol and aqueous) rich in phenolic compounds exhibited excellent antioxidant activity in terms of DPPH radical scavenging, ferric chloride reducing power and lipid peroxidation inhibition in rat liver homogenate [9]. The antioxidant activity depends on the chemical characteristics of each compound and the model system used, a given compound can show antioxidant activity in liver and brain homogenates but may fail to give a clear response when provided in the diet or given by gavage, because several factors can interfere *in*

*in vivo* such as absorption, transport and metabolism of the drug by the organism. Therefore, an *in vivo* study was planned without using any chemical oxidative stress inducers.

The findings of the study also support our earlier findings, wherein, supplementation aqueous extract of the bark resulted in a significant reduction ( $p \leq 0.01$ ) of TBARS and significant enhancement ( $p \leq 0.01$ ) of GSH content in serum, kidney and livers of streptozotocin-induced diabetic rats compared to untreated diabetic rats [14]. In chronic toxicity studies no significant differences ( $p \leq 0.05$ ) were observed between the TBARS and GSH levels in the extract treated and untreated groups [15]. Hence, the *in vivo* antioxidative activity of FRSACE was determined as a reduction in spontaneous lipid peroxidation of brain homogenate by the addition of plasma *ex vivo*.

### Conclusion

In conclusion, it is inferred that the *F. racemosa* bark extract possesses significant antioxidant activity *in vivo* and its antioxidant activity is mediated by decreasing thiobarbituric acid reactive substances and increasing glutathione concentrations in the tissues.

### Conflict of Interest

Authors do not have any conflicts of interest.

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