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Antioxidant Activity of Ethanolic Extract of *Euphorbia Thymifolia* Linn.

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The antioxidant activity of the ethanol extract of the whole plant of *Euphorbia thymifolia* Linn. has been evaluated in both *in vivo* and *in vitro* experimental models, by estimating the malondialdehyde content of rat brain, which is one of the products of the lipid peroxidation. The ethanolic extract of the plant showed significant inhibition of lipid peroxidation level comparable to that of Vitamin E used as standard.

Euphorbia thymifolia Linn. (Euphorbiaceae) is a small prostrate, hispidly pubescent, annual weed with numerous horizontally spreading branches occurring in red and green form. It is native of India but also distributed throughout the tropics except North Australia. The leaves, seeds and fresh juice of the whole plant are reported to be used in worm infections, as stimulant and for astringent actions². The plant is reported to contain a large number of phenolics³. As a great deal of interest is being directed towards the bioactivity of the phenolics as dietary source of anti oxidants, it was thought worthwhile to evaluate the antioxidant properties of *E. thymifolia* also, which is rich in phenolics.

The basis of experimental model is the measurement of the amount of malondialdehyde (MDA), which is one of the free radical induced lipid peroxidation products of rat brain phospholipids. MDA forms a colored adduct with thiobarbituric acid (TBA) and are measured colorimetrically. In case of *in vitro* study, FeCl₃ and ascorbic acid has been used for hydroxyl radical (OH[•]) generator while, CCl₄ has been used in *in vivo* study for the generation trichlorocarbon radical (CCl₃[•]).

The plant (*E. thymifolia*) was collected from Tamilnadu and was duly authenticated at the Sugavanam Herbal Center, Pudukkottai. A voucher specimen (No. PCRL 36) has been deposited in the Department of Pharmaceutics, BHU. The air dried whole plant was coarsely powdered

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TABLE 1: *IN VIVO* LIPID PEROXIDATION ASSAY

Groups	Treatment	Lipid peroxidation level (n mol MDA in 100 mg Brain tissue)
A	Vehicle	957.7±2.605
B	CCl ₄	2159±1.01
C	Vitamin E + CCl ₄	1223±2.50
D	Ethanol extract of <i>E.thymifolia</i> + CCl ₄	1203±2.60

All values are expressed as mean±SD, n=6; P<0.001 for all groups, A vs. B, B vs. C, B vs. D, C vs. D, and vice versa. The calculations were made by student t- test

(600g) and extracted with petroleum ether (60-80°, 2.5 l, 24 h) and ethanol (2 l, 24 h) successively in a Soxhlet apparatus. The ethanol extract of *E. thymifolia* which showed the presence of large number of phenolics/tannoids, and has been used for evaluation of its antioxidant activity.

Wistar rats of either sex (100-200 g), procured from Zoological emporium, Varanasi, were housed in colony cages and maintained on a 12 h light/dark cycle at an ambient temperature. The animals were allowed access to Lipton feed (Lipton India limited, Bangalore) and water *ad libitum*. The animal experiments were approved by the institutional animal ethics committee.

In the *in vivo* study, the estimation was made using the method of Okhawa *et al.*⁴. The Wistar rats were divided into four groups, each group consisting of six animals. These groups were treated as follows: standard (vitamin-E: 50 mg/kg), and the ethanolic extract of *E. thymifolia* (100 mg/kg) respectively for seven days (Table 1).

In the *in vitro* study, the estimation was made using the method reported by Braughler *et al.*⁵ with a slight modification. The rat brain was promptly excised after decapitation, weighed and washed with ice-cold KCl solution (1.15%), blotted with filter paper and sliced. Rat brain homogenate was prepared in the ratio of wet brain tissue (0.75 g) to KCl solution (1.15%, 9.25 ml) using a glass homogenizer. Increasing concentrations (0.1 to 1 ml) of rat brain homogenate were taken in clean test tubes. Sodium lauryl sulphate (SLS, 8.1%, 0.2 ml) was added to all the test tubes and were shaken well. FeCl₃ (0.25 ml, 0.25 mM) and ascorbic acid (0.25 ml, 1 mM) were added in the test tubes, shaken well, incubated at 37° for 30 min.

TABLE 2: EFFECT OF ETHANOLIC EXTRACT OF *E. THYMIFOLIA* ON LIPID PEROXIDATION *IN VITRO*

Concentration of ethanolic extract of <i>E. thymifolia</i> (µg)	% of lipid peroxidation
0	0
100	16.7±0.006
200	25.6±0.04
300	42.7±0.01
400	56.0±0.04
500	66.7±0.01
600	76.3±0.003
700	88.2±0.03
800	92.0±0.07
900	92.0±0.07
1000	92.0±0.07

The data is presented as mean ±SD, n=6

Thereafter, acetate buffer (pH-3.5, 1.5 ml) and TBA solution (0.8%, pH- 7.4, 0.5 ml) were added in succession. The volume was adjusted to 10 ml with doubled distilled water. The mixture was heated at 85° for 30 min and centrifuged with n butanol-pyridine mixture (15:1 v/v, 5 ml) at 4000 rpm for 10 min⁶. The organic layer was taken by decantation and absorbance was measured at λ 532 nm by UV spectrophotometer (Jasco Model, 7800). A suitable concentration (0.5 ml) showing good absorbance was selected to study the effect of the ethanolic extract of *E.*

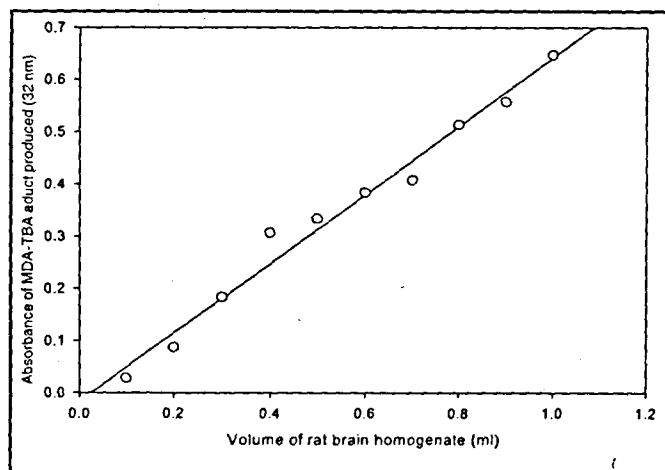


Fig. 1: Lipid peroxidation assay

thymifolia (fig.1). The above procedure was followed by taking 0.5 ml of rat brain homogenate with different concentrations (0-1000 µg) of ethanolic extract of *E. thymifolia* (Table 2).

The effect of ethanolic extract of *E. thymifolia* on lipid per oxidation level, both *in vivo* and *in vitro* is summarized in Table 1 and 2. The results obtained indicate that the ethanolic extract of *E. thymifolia* has significantly reduced the free radical induced lipid peroxidation of rat brain phospholipids in both *in vivo* and *in vitro* experiments. In case of *in vivo*, it was found to be comparable to vitamin E, while in *in vitro* study a dose-dependant response at lower doses was observed.

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Modulated Permeation of Insulin through Glucose Sensitive Membrane

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Glucose sensitive membrane was prepared by solution casting method containing pH sensitive microparticles. The membranes consisted of semiinterpenetrating polymer network microparticles of poly(vinyl alcohol) and poly(methacrylic acid), Glucose oxidase and catalase enzyme. The immobilized glucose oxidase acted as the glucose sensor and catalyzer; it was sensitive to glucose and catalyzed the glucose conversion to gluconic acid. Catalase was used to convert hydrogen peroxide to oxygen. There was no detectable leakage of enzymes in the release media and the activity of the immobilized enzymes was 70% compared to that of free enzymes. The permeability of insulin was dependent on the glucose concentration. The insulin permeability increased 3 times when the glucose concentration was increased from 50 mg% to 200 mg% and increased by 7 times when the glucose concentration was 400 mg%.

Diabetes mellitus is a major cause for the death in industrialized countries and multiple parenteral injection of insulin are currently the standard treatment for insulin dependent diabetic patient. A variety of degenerative

conditions observed in diabetics such as neuropathy and increased vascular disease may stem from the poor control of the blood glucose level due to subcutaneous injections^{1,2}. Of the various delivery techniques developed for insulin delivery, self-regulated insulin delivery systems have attracted growing interest due to the presence of both glucose sensing and insulin delivery functions. The self-

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