RESEARCH PAPER

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Lipid Peroxidation Inhibition and Anti-Anoxic Property of 1- Phenyl-3-(4-Hydroxy-3, 5-Ditertbutylphenyl), Prop-2-En-1-One

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A Phenyl Styryl ketone, with sterically hindered phenolic group, 1-phenyl-3-(4-hydroxy-3,5-ditertbutylphenyl), prop-2-en-1-one (PHP) was evaluated as *in vitro* inhibitor of iron-ascorbate stimulated lipid peroxidation in rat brain homogenates. It was found to be more potent than the standard antioxidant vitamin E. Further PHP was evaluated for its effect in vivo against nitrogen-induced anoxia. The test compound protected the mice significantly against nitrogen-induced anoxia.

THE inherent biochemical, anatomical and physiological characteristics of the brain make it especially vulnerable to oxidative insult[1]. When oxygen supply to the brain becomes deficient, the energy metabolism is affected. This results in many events including massive release of excitatory neurotransmitters and influx of calcium, which finally leads to the degradation of cells and tissues[2]. Reactive oxygen species are implicated in many CNS disorders like hypoxia, ischemia, trauma, stroke and transition metal-dependent reactions[1-6]. Inhibitors of lipid peroxidation are known to be useful in CNS trauma and anoxic conditions. Aminosteroids are a novel class of lipid peroxidation inhibitors with significant activity against CNS trauma and KCN-induced hypoxia[7-9]. Many derivatives of 4-arylpyrimidines[10] and 11-[4- (cinnamyl)-1-piperazinyl]-6,11-dihydrodibenz [b,e]oxepin[11] with anti-lipid peroxidation activity, have been shown to have anti-anoxic activity.

Earlier studies have shown that many styryl ketones like curcumin[12-14], dehydrozingerone[12,15,16] are potent antioxidants with free radical scavenging and lipid peroxidation inhibiting activities. In a search for more potent antioxidants, we have studied a number of phenyl styryl ketones[17]. The present study shows that the title compound with a sterically hindered phenolic group (**fig. 1**) has potent antioxidant activity in rat brain homogenates. The compound was also investigated for its protection against nitrogen-induced normobaric anoxia.

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Materials and methods

(±)-a-Tocopherol, butylated hydroxy toluene (**BHT**), thiobarbituric acid (**TBA**) were obtained from Sigma chemical Co., USA. All other reagents were of the highest grade available commercially. 1-phenyl-3-(4-hydroxy-3,5- ditertbutylphenyl), prop-2-en-1-one (**PHP**) was synthesized essentially by the method reported earlier[18] and the structure was confirmed by spectral (NMR and IR) studies. The purity of the compound was tested by elemental analysis, m.p. and by TLC.

Albino Charles-Foster rats (180-200 g) of either sex were used for the **in vitro** studies. Male Swiss albino mice weighing 25 to 30 g were used to study the effect of the test compound on normobaric anoxia and in acute toxicity studies.

Inhibition of lipid peroxidation

Lipid peroxidation inhibition by test compounds in rat brain homogenates (10%, w/v) was studied according to the method previously reported[16]. In brief, the incubation mixture contained in a final volume of 1 ml, brain homogenate (500 ul), KCl (150 mM) and ethanol (10 ul) or test compound dissolved in ethanol. Ferric chloride (100 uM) was added to the incubation mixture and peroxidation was initiated by adding ascorbic acid (100 uM). After incubating for 20 min at 37°C, the reaction was stopped by adding 2 ml ice and cold 0.25 N HCl containing 15% trichloroacetic acid, 0.38% thiobarbituric acid and 0.05% BHT. Following heating at 80°C for 15 min, samples were cooled, centrifuged at 1000 x g for 10 min and the absorbance of the supernatant was measured at 532 nm. The amount of lipid peroxidation was determined using the molar extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$ and expressed as thiobarbituric acid reactive substances (TBARS) as described by Braughler **et al**[19]. Percent inhibition of TBARS formed was calculated by comparing with vehicle only control experiments. Results are the mean of one individual experiment conducted in triplicate.

Normobaric hypoxia

The experiment was carried out by a method reported previously[10,20]. In brief, the effect of **PHP** on the survival time mice subjected to anoxia (100% Nitrogen) was studied by maintaining two mice in a closed glass chamber in which a current of nitrogen gas was circulated, and their survival time was measured. One mouse was pre-treated intraperitoneally with the **PHP** suspended in 0.5% ethyl cellulose, and the other with the vehicle 30 min before the experiment. Results are expressed as mean time of survival ± S.E.

Acute toxicity

The acute toxicity was studied in mice by administration of **PHP** intraperitoneally at doses of 250, 500 and 1000 mg/kg body weight. Each group consisted of 10 mice and were observed for mortality over a period of seven days, after receiving the test compound.

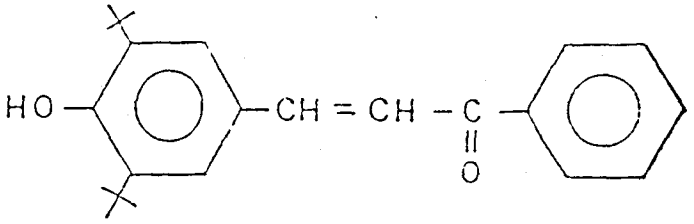

Fig.1: Structure of 1-phenyl-3-(4-hydroxy-3, 5-ditertbutyl phenyl), prop-2-en-1-one

RESULTS AND DISCUSSION

The antioxidant property of **PHP** was studied by assessing the inhibition of lipid peroxidation stimulated by Fe^{3+}- ascorbate in rat brain homogenates. Ferric ions undergo reduction and initiates the process of lipid peroxidation[21]. In the control experiments, the amount of TBARS formed was 27.4 nmoles/ml of the tissue homogenates, when stimulated by Fe^{3+}-ascorbate. In all these experiments, BHT was added after the incubation but before heating. This prevents the formation of additional TBARS during the heating due to the breakdown of the lipid peroxides[19].

Control experiments showed that **PHP** did not affect the measurement of TBARS (omission of the brain homogenate from the reaction mixture abolished chromogen formation). Also **PHP** did not interfere with the TBA test, since it did not alter the color development if added at the end of the incubation, but before heating. All the tests were carried out in triplicate and the variation within the experimental set was found to be less than 5 percent.

The inhibitory effect of **PHP** on lipid peroxidation was studied at various concentrations (**fig. 2**). **PHP** was found to be more effective than vitamin E at

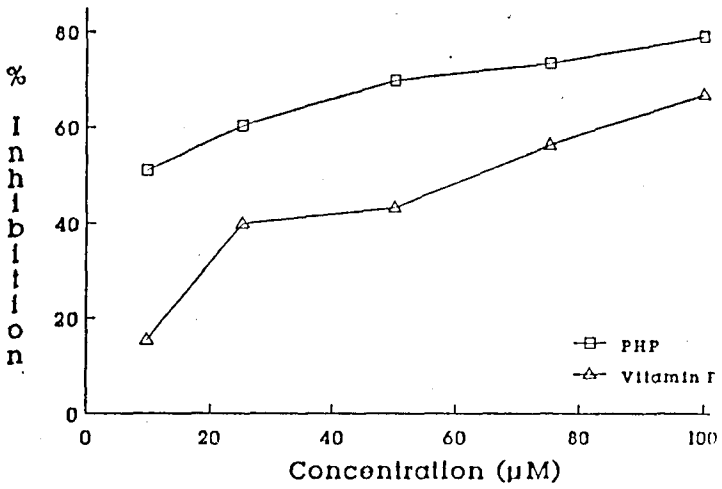

Fig. 2: Effect of test compounds on lipid peroxidation induced by ferric-ascorbate in rat brain homogenate Ferric (100 um)-(ascorbate (100 uM) was added to incubation mixture containing brain homogenate (500 ul), test compounds in ethanol (10 ul) and KCl (150 mM) in a final volume of 1 ml.
TBARS formed was measured at the end of 20 min incubation and % inhibition by test compounds was calculated in comparison with diluent only control experiments.
PHP: 1-phenyl-3-(4-hydroxy-3, 5-ditertbutylphenyl), prop-2-en-1-one.

all the concentrations tested. The IC_{50} values was calculated from the regression equation based on inhibition of lipid peroxidation by test compounds at five different concentration. The IC_{50} for **PHP** was found to be 9.7 uM. It was about five times more potent than the naturally occurring antioxidant, vitamin E (IC_{50} = 52.0 uM).

PHP was also investigated for its protective activity against nitrogen-induced anoxia. Male mice were pre-treated intraperitoneally with the test compound or vehicle, 30 min before the experiment. The animals in a pair (one vehicle and other compound treated) was kept in a closed glass chamber, in which a stream of nitrogen was circulated. The survival time of the mice was given in **Table 1. PHP** at both the doses tested protected the animals significantly against normobaric anoxia.

Table 1 - Effect of test compound on survival time of mice subjected to nitrogen-induced anoxia

Treatment	Sample size (n)	Survival time (min)
Control (Vehicle)	10	14.70 ± 0.94
PHP		
100 mg/kg	5	24.60 ± 1.03
200 mg/kg	5	36.00 ± 1.41

The test compound was administered intraperitoneally 30 min before the experiment. Anoxia was induced as described under materials and methods. Values represent mean survival time ± S.E.

PHP: 1-phenyl-3-(4-hydroxy-3,5-ditertbutylphenyl), prop-2-en-1-one.

In acute toxicity experiments in mice, it was found that **PHP** has not produced any mortality upto a maximum of 1000 mg/kg given intraperitoneally, indicating that i.p. LD_{50} of **PHP** is greater than 1000 mg/kg.

Several antioxidants are known to show protection against KCN- induced hypoxia[9] and nitrogen-induced anoxia[10,11]. Compounds related to **PHP**, having a 3,5-diterbutylphenol nucleus have been studied extensively as antioxidants and are used for the treatment of stroke[22-24]. It has been long recognized that the mitochondria, rich in phospholipids containing unsaturated fatty acids, play a crucial role in maintaining energy metabolism. Free radical reactions of mitochondrial phospholipids occur under normal physiological conditions. However, in ischemic conditions, there is an excessive production of free radicals leading to the peroxidation of the phospholipids, causing changes instructure and function of the mitochondrial membrane, and brain mitochondria are especially vulnerable to such ischemic attacks[3,10]. **PHP** being a potent inhibitor of lipid peroxidation may be preventing the mitochon-

drial lipid oxidation and thus exhibit protection against nitrogen-induced anoxia. Further **PHP** also posses antiinflammatory activity and inhibits the prostaglandin synthetase activity[18]. Thus the present study shows that 1- phenyl-3-(4-hydroxy-3,5-diterbutylphenyl), prop-2-en-1-one possess potent antioxidant and anti-anoxic properties.

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