Betel leaf extract can be both antiperoxidative and peroxidative in nature

Piper betle Linn. of family Piperaceae is a perennial dioecious creeper, whose leaves are widely used as masticatory. They are claimed to be digestive stimulant and carminative[1]. These leaves are also attributed with some medicinal properties such as being antipoisonous, wound healing, antiseptic in diptheria, resolvant to glandular swellings, antispermatogenic and effective in bronchial asthma[2,3]. We have attempted to evaluate its dose-dependent effects, hitherto uninvestigated on tissue lipid peroxidation (LPO). Superoxide dismutase (SOD) and catalase (CAT) activities were also studied to correlate with the peroxidative changes, if any.

Mysore variety of betel leaves were collected locally. The leaf extract was prepared according to the method of Prabhu *et al.*[4]. In brief, fresh betel leaves were taken, washed and known quantity was thoroughly ground to a paste in a mortar and then homogenized in a Potter–Elvehjem homogenizer and made up to a known volume. Four doses: A, 0.1 g/kg body wt; B, 0.4 g/kg body wt; C, 0.8 g/kg body wt and D, 2 g/kg body wt were prepared in distilled water for the treatment.

Thirty five adult healthy colony-bred Swiss albino male mice weighing 30 ± 2 g were maintained in a constant temperature (27 ± 1°C and light 14L:10D) controlled room with the provision of food (Hindustan Lever Ltd., India) and water *ad libitum*. Seven days after acclimatization, the mice were divided into five groups of seven each. Initial body wt of each animal was recorded. Group I received 0.1 ml/day of normal saline and served as control. Groups II, III, IV and V were treated with betel leaf extract, of 0.1 g, 0.4 g, 0.8 g and 2.0 g/kg body wt/day respectively. The experiment was continued for 15 days.

On the last day of the experimentation, the final body weight of each animal was taken. After exsanguination, the liver and kidney were removed quickly, washed with phosphate-buffered saline (PBS, pH 7.4). Liver was processed immediately for biochemical estimations. LPO was determined by the reaction of TBA with malondialdehyde (MDA), a product formed due to the peroxidation of lipids, following the method of Ohkawa *et al.*[5]. The amount of MDA formed was measured by taking the absorbance at 532 nm using a Shimadzu UV-160 A spectrophotometer. Hepatic SOD activity was estimated by measuring the percentage inhibition of the pyrogallol autoxidation by SOD according to the method of Marklund and Marklund[6]. One unit of SOD was defined as the enzyme activity that inhibits the autoxidation of pyrogallol by 50%. Catalase activity was estimated following the method of Aebi[7] and was expressed as μM of H_2O_2 decomposed per min per mg protein. The hepatic protein content was determined by the method of Lowry *et al.*[8].

The results are summarized in Figures 1–3. A significant increase in hepatic LPO (Figure 1) was observed in two higher-dosed groups. While no change was found in 0.4 g/kg treated group, it

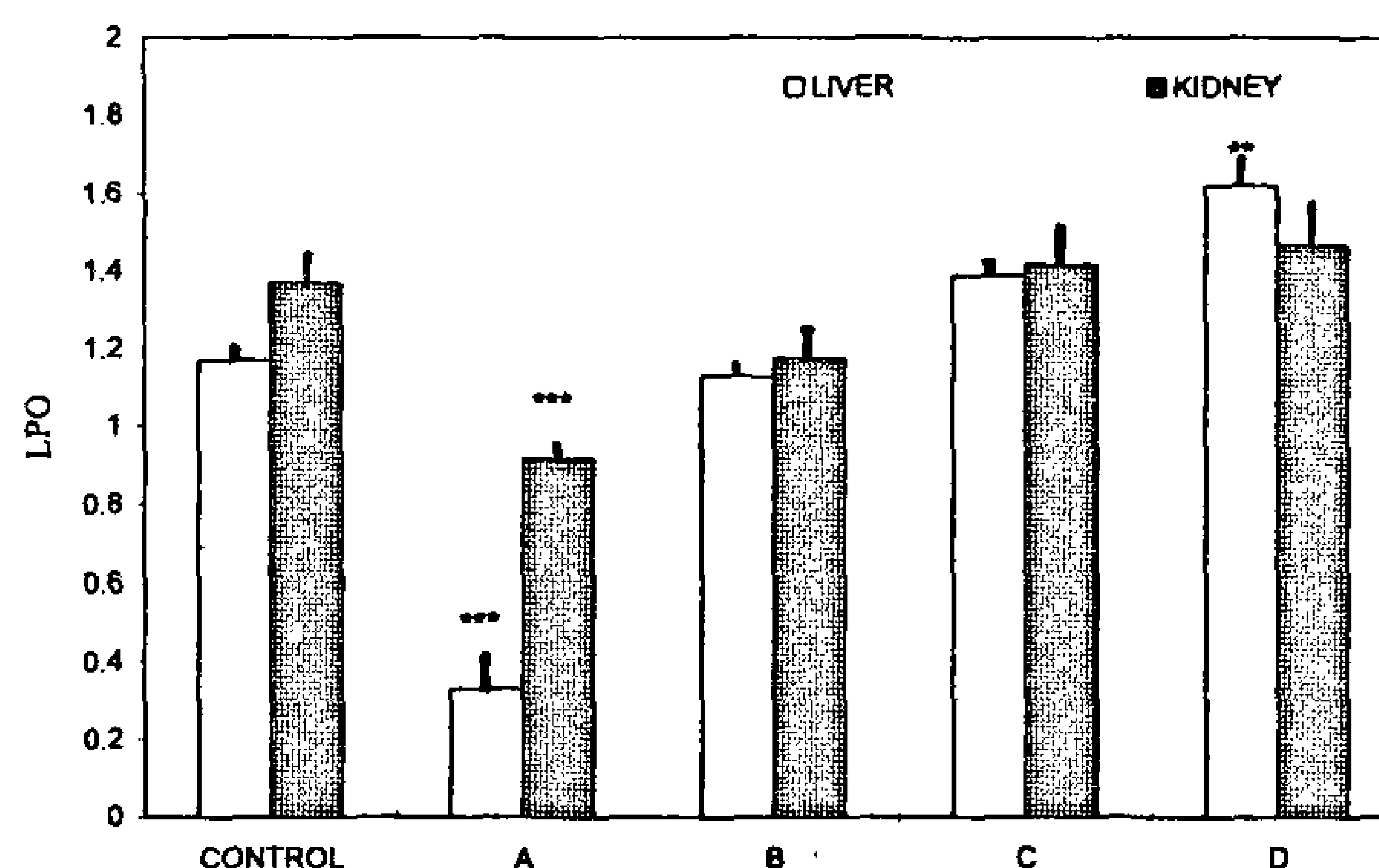

Figure 1. Effects of different doses (A, 0.1 g; B, 0.4 g; C, 0.8 g and D, 2.0 g/kg/day) of betel leaf extract for 15 days on hepatic and renal LPO (nM MDA formed/h/mg protein) in male mouse. *, $P < 0.05$; **, $P < 0.01$ and ***, $P < 0.001$ compared to the respective control values.

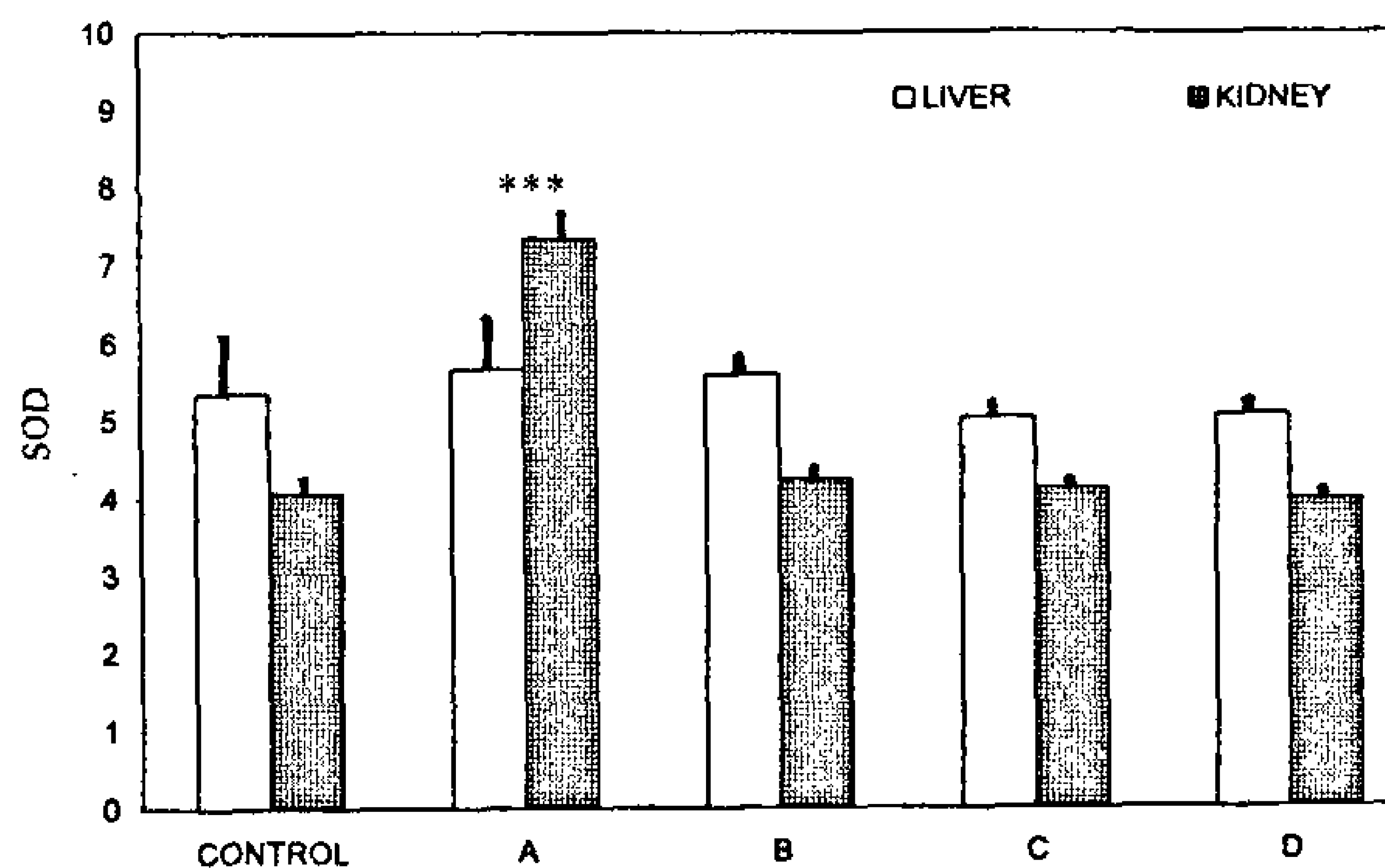

Figure 2. Effects of different doses (A, 0.1 g; B, 0.4 g; C, 0.8 g and D, 2.0 g/kg/day) of betel leaf extract for 15 days on hepatic and renal activity of SOD (units/mg protein) in male mouse. ***, $P < 0.001$ compared to respective control value.

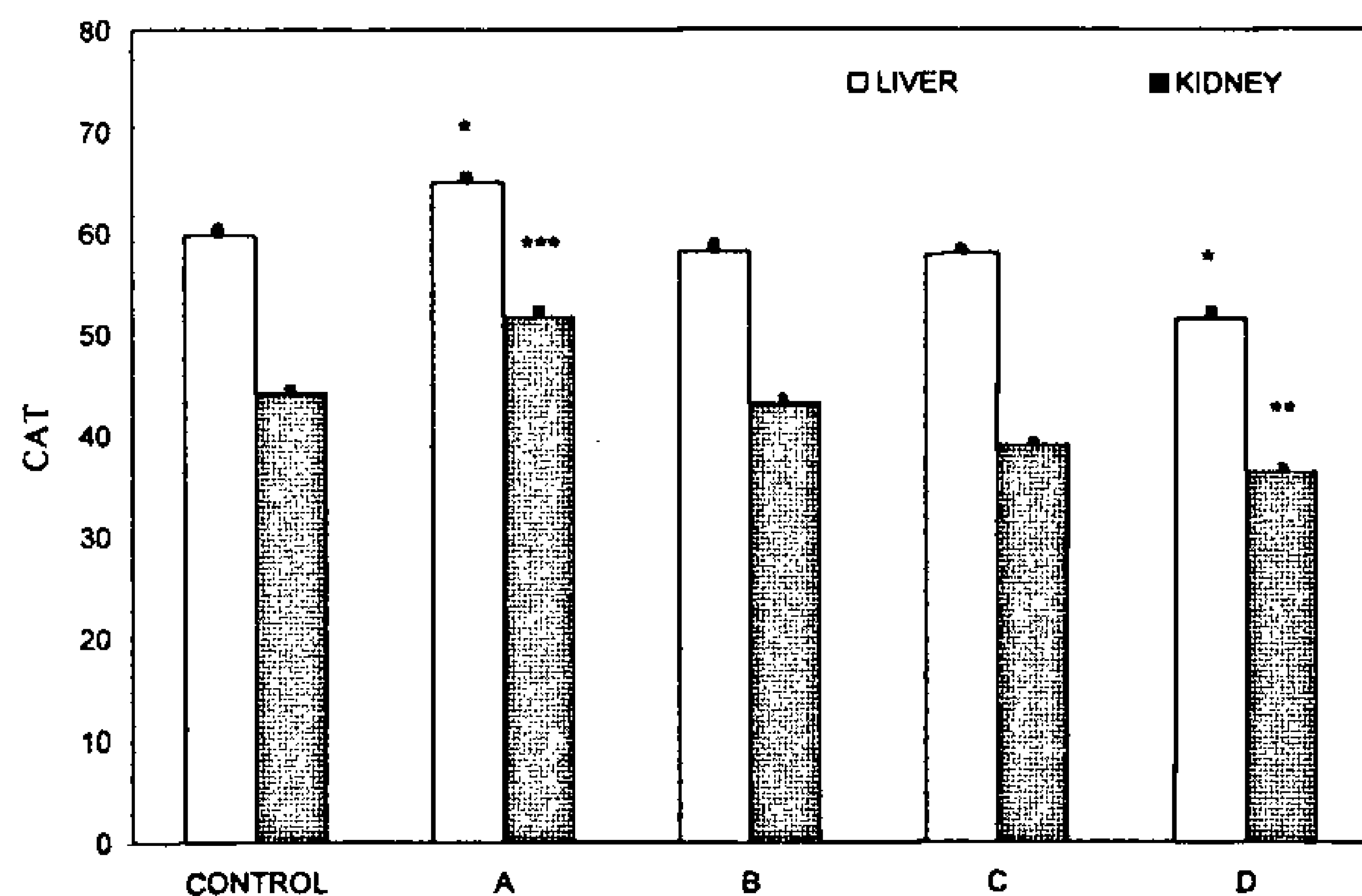

Figure 3. Effects of different doses (A, 0.1 g; B, 0.4 g; C, 0.8 g and D, 2.0 g/kg/day) of betel leaf extract for 15 days on hepatic and renal activity of CAT (μmole of H_2O_2 decomposed/min/mg protein). *, $P < 0.05$, **, $P < 0.01$ and ***, $P < 0.001$ compared to the respective control values.

was significantly low in 0.1 g/kg body wt dosed group. Almost parallel effects were observed in kidney homogenate. Hepatic SOD activity remained unaltered in all the three treated groups except in kidney homogenate where a significant increase was observed only in lowest dosed group. While CAT activity was significantly increased in lowest dosed group, it was significantly decreased by the highest dose. Changes in body weight were not significantly different in any of the treated groups compared to that of control.

From the results it is evident that, betel leaf extract exhibits dose-dependent actions. At low concentration it is antiperoxidative, whereas at higher concentrations it induces oxidative damage to biological membranes as indicated by the enhanced LPO in liver and kidney tissues, supported by a concomitant decrease in tissue CAT activity. The lowest dose (0.1 g/kg body wt) inhibited LPO both in liver and kidney tissues, indicating antiperoxidative/protective effect of betel leaf extract. Similar to the present finding, the antiperoxidative effect has also been reported in ashwagandha by us[9]. Although extract of two other plants[10,11] are also known to be antiperoxidative in nature, dose-dependent differential effects were not found in these plants as presently observed in betel leaf extract.

Lipid peroxidation is an oxidative deterioration of polyunsaturated fatty acids (PUFA) and is usually initiated by free radicals[12]. Most of these radicals are destroyed by SOD. However, superoxide anion (O_2^-) reacts with water to form H_2O_2 which in turn is responsible for the generation of hydroxyl radicals, OH that attack membrane fatty acids inducing LPO. CAT is believed to be the most effective defensive agent against high concentration of H_2O_2. Interestingly in the present study, higher concentration of the drug decreased and the lowest concentration increased the CAT activity, suggesting that dose dependent differential actions of the plant extract on LPO are probably CAT mediated.

Our findings, clearly suggest that excess consumption of betel leaf may prove harmful to the body, while low amount could be beneficial. However, further investigation(s) involving long-term experiments are required to reveal more interesting facts on this aspect.

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