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Full Length Research Paper

Piper sarmentosum Roxb protects lungs against oxidative stress induced by carbon tetrachloride in rats

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This study was designed to investigate the effects of *Piper sarmentosum* supplementation on carbon tetrachloride (CCl_4) induced oxidative stress in lung. Thirty-two male Wistar rats were divided into two groups; a control and a treatment group which was given 125 mg/kg body weight *P. sarmentosum* orally for 28 days, after which each group was further subdivided into two groups. A group was exposed to CCl_4 (1 ml/kg body weight, orally), while another group was given corn oil. After 24 h, blood and lung were taken. CCl_4 increased lung lipid peroxidation (TBARS) and glutathione peroxidase significantly. *P. sarmentosum* supplementation was able to reduce these increases. However, both CCl_4 and *P. sarmentosum* extract did not affect lung superoxide dismutase activity. In conclusion, *P. sarmentosum* is capable in reducing the oxidative stress in lungs by decreasing lipid peroxidation and maintaining the glutathione peroxidase activity towards the normal level.

Key words: *Piper sarmentosum*, oxidative stress, antioxidant, carbon tetrachloride.

INTRODUCTION

The genus of *Piper* was distributed in the tropical region especially in South East Asia country like Malaysia, Thailand and Indonesia. *Piper sarmentosum* which is one of the Piparaceae family, locally known as 'kaduk', is a herb plant that has a wide usage in traditional medicine. It has been used widely in folk medicine especially in Southeast Asia region but very few studies have been documented scientifically.

Some studies have shown that this tropical plant possesses medicinal properties. It was reported to have anti-amoebic effect against *Entamoeba hystolytica* at high dose (Sawangjaroen et al., 2004), antituberculosis, antiplasmodial (Rukachaisirikul et al., 2004), neuro-muscular blocking (Ridtitid et al., 1998) and hypoglycemic activities (Peungvicha et al., 1998). *P. sarmentosum* was found to possess high antioxidant activity and it might be attributed to the chemical components present in the plant such as vitamin C and E, xanthophylls, carotenes and phenols (Chanweethesuk et al., 2005).

Carbon tetrachloride (CCl_4) is widely used as model to produce liver injury in many studies (Hwang et al., 2007; Lim et al., 2008; Fu et al., 2008). CCl_4 administration has also been demonstrated to cause injury to the lungs (Mizuguchi et al., 2006; Zhang et al., 2007). It is proven that CCl_4 promotes injuries in these organs via oxidative stress by increasing the lipid peroxidation and lowering the endogenous antioxidant (Hwang et al., 2007; Fu et al., 2008).

Common diseases in the lung such as asthma, chronic obstructive pulmonary disease and cystic fibrosis had been demonstrated to share similar pathogenesis, that is, increased production of reactive oxygen species and this had been shown to correlate with the disease severity (Kuleci et al., 2008; Markart et al., 2009; Suzuki et al., 2008). Antioxidant defense in the lungs including glutathione peroxidase and superoxide dismutase had also been reported to be reduced in patients with asthma

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(Sackesen et al., 2008).

There is relatively little information pertaining to the antioxidant activity of *P. sarmentosum* and its possible use to inhibit oxidative stress in tissues. Since the leaves are one of the commonly used spices, it is therefore of interest to investigate the effects of its methanolic extract on CCl_4-induced oxidative stress in rat lungs.

MATERIALS AND METHODS

Animals and experimental design

The male Wistar rats weighing between 180 to 200 g, used in this study were obtained from the Laboratory Animal Resource Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia. All rats were kept on a regular night/day cycle, with natural light for a period of 12 h (0700 to 1900 h). Throughout the feeding period, all rats were habituated to handling to reduce their stress-related disturbances. The rats were housed in large cages with wide wire-mesh bottoms to prevent coprophagy. Food and water were given *ad libitum* throughout the experiment. The Animal Care and Use Committee (ACUC) of the Faculty of Medicine, Universiti Kebangsaan Malaysia, had approved this study (Approval number: FAR/2006/AZLINA/12-AUGUST/129). All chemicals and enzymes were obtained from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise stated.

Thirty-two rats were divided into two equally sized groups. The control group was fed with normal rat chow (RC) while the treatment group received the same diet with oral supplementation of *P. sarmentosum* at the dose of 125 mg/kg body weight for 28 days. The dose selected was based on previous study by Peungvicha et al. (1998). *P. sarmentosum* was dissolved in dimethyl sulfoxide (DMSO) and corn oil (15:85) which acts as a vehicle. It was administered by oral gavage using an 18G gavage needle. The control groups were sham administered with the vehicle. At the end of treatment period, each group were further divided into two subgroups of 8 rats; a group was exposed to CCl_4 (1 ml/kg body weight orally), while another group was only given corn oil. The CCl_4 dose was selected based on our preliminary study, where at the dose of 1 ml/kg, a significant oxidative damage to the lung was observed. The CCl_4 was prepared based on the method by De Zwart et al. (1997). Twenty-four hours after exposure to CCl_4, blood samples were taken via orbital sinus under diethyl ether anesthesia and the rats were killed. Lungs and plasma samples were taken for the measurement of thiobarbituric acid reactive substances (TBARS) level, superoxide dismutase and glutathione peroxidase activities.

Plant extraction

The *P. sarmentosum* leaves were collected from the Forest Research Institute of Malaysia (FRIM) and extracted following a method previously described by Sawangjaroen et al. (2004). A voucher specimen has been deposited at the institute (FRI 45870). The yield of the dried extract was 6% of the fresh leaves.

Biochemical parameter determinations

The lipid peroxidation content measured as thiobarbituric acid reactive substances (TBARS) in the lung was determined using the method described by Ledwozyw et al. (1986). The protein concentration was determined by the Lowry et al. (1951) method using bovine serum albumin as the standard. The activities of glutathione peroxidase (Lawrence and Burk, 1976) and superoxide dismutase (Beyer and Fridovich, 1987) were analyzed according to the described methods.

Statistical analysis

Results are expressed as mean ± SEM. Statistical significance ($P<0.05$) was determined by ANOVA followed by post hoc Tukey test. A P value less than 0.05 was considered statistically significant.

RESULTS

Lung TBARS

Carbon tetrachloride (CCl_4) administration increased lung TBARS significantly ($p = 0.014$), as shown in Figure 1. In the *P. sarmentosum* -supplemented group and exposed to CCl_4, the lung TBARS was lower compared to non-supplemented group. Its TBARS level was also similar to the non-exposed groups (supplemented and control). In the groups not receiving CCl_4 (*P. sarmentosum* and control), the TBARS levels were not different ($P>0.05$).

Plasma TBARS

Figure 2 shows the effect of *P. sarmentosum* on CCl_4-induced lipid peroxidation in plasma measured as TBARS. CCl_4 administration did not affect the plasma TBARS significantly in all groups. *P. sarmentosum* supplementation for 28 days also had no effect on this parameter.

Lung glutathione peroxidase activity

CCl_4 increased glutathione peroxidase enzyme activity in the lungs. *P. sarmentosum* supplementation significantly reduced the enzyme activity after exposure to CCl_4 (Figure 3) and its activity was not significantly different from the non-exposed control and its own non-exposed group.

Lung superoxide dismutase activity

As shown in Figure 4, the activity of lung superoxide dismutase in rats exposed to CCl_4, with or without supplementation of *P. sarmentosum.* The lung superoxide dismutase was not significantly affected by both CCl_4 and *P. sarmentosum*.

DISCUSSION

Medicinal plants used at current are mainly based on

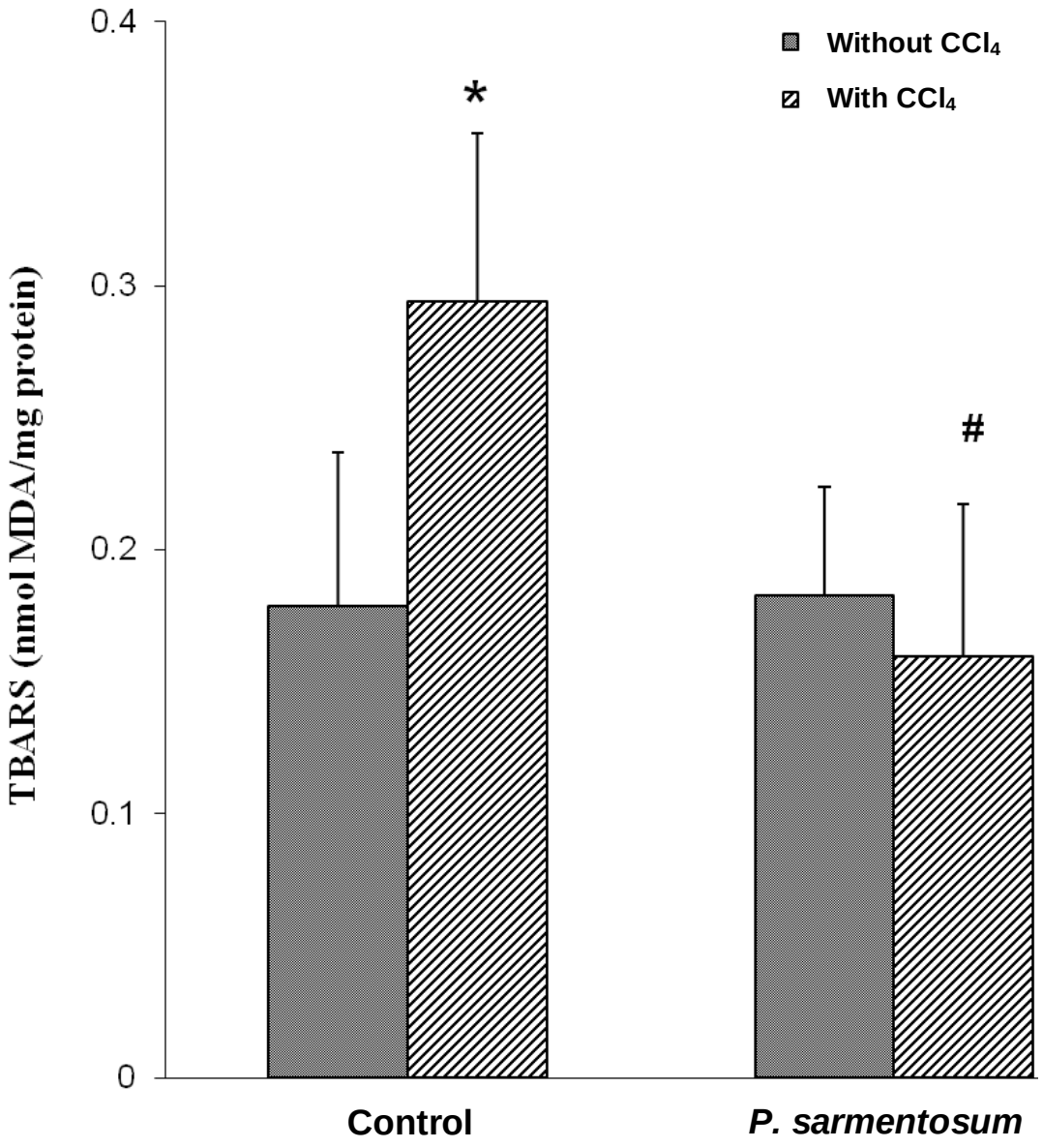

Figure 1. The effect of *Piper sarmentosum* supplementation (125 mg/kg body weight, orally) for 28 days on lung thiobarbituric acid reactive substances (TBARS) levels in rats exposed to CCl_4 (1 ml/kg body weight orally). Bars represent mean ± SEM (n = 8). *Significantly different compared to non-exposed control ($p<0.05$). #Significantly different compared to the exposed control ($p<0.05$).

experience of many generations of traditional ethnic systems of herbal medicine. Although widely used or consumed, the scientific evidence is lacking and more research are warranted. In Malay traditional society, water decoction of the leaves are being used for the treatment of cough, headache, back pain and arthritis, while the water decoction of the roots are been used in treatment of menstrual pain and to improve urination.

In this study, the increase in TBARS induced by CCl_4 was prevented by the supplementation of *P. sarmentosum*. This finding proved that the methanolic extract of *P. sarmentosum* possesses antioxidant property as claimed by Chanweethesuk et al. (2005). They reported that the plant contains high concentration of antioxidants such as vitamins C, E and phenols. Whether the antioxidant property of the extract contributed by the antioxidants present, or its own chemical properties, it will need further investigation. The similar level of TBARS in the supplemented group that was not exposed to CCl_4, indicated that *P. sarmentosum* was not toxic to the lungs. Hussain et al. (2010) reported a similar finding in the liver where preservation of

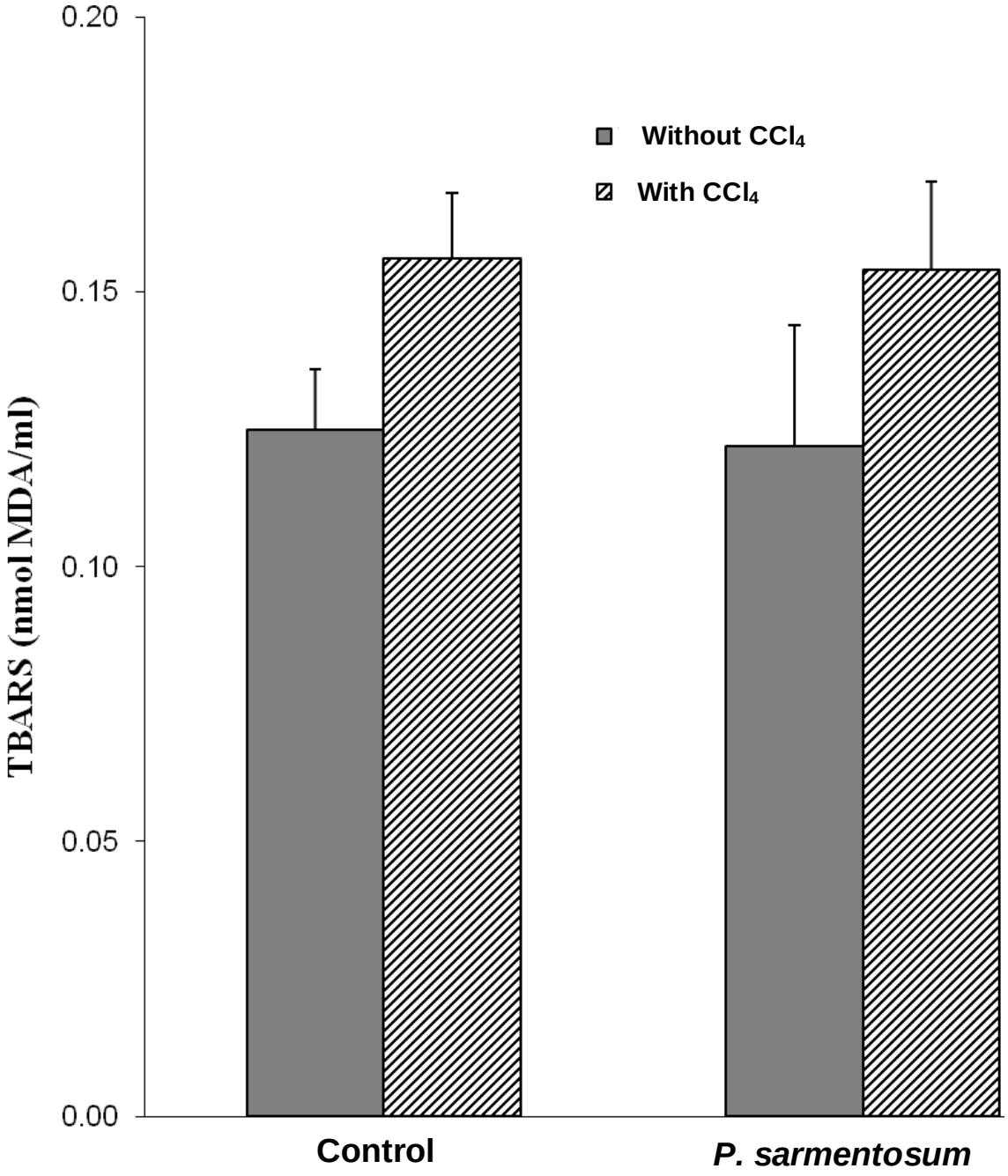

Figure 2. The effect of oral CCl_4 (1 ml/kg body weight) on plasma thiobarbituric acid reactive substances (TBARS) levels in *Piper sarmentosum*-supplemented (125 mg/kg body weight, 28 days, orally) rats. Bars represent mean ± SEM (n = 8). No significant differences were seen between groups.

markers of antioxidant activity, total plasma antioxidant activity (TPAA), total protein (TP), superoxide dismutase (SOD), catalase (CAT), and thiobarbituric acid reactive species (TBARS) were observed with pretreatment of *P. sarmentosum*.

Other studies had also demonstrated increased lipid peroxidation in lungs after exposure to CCl_4 (Mizuguchi et al., 2006; Zhang et al., 2007). CCl_4 is converted into trichloromethyl radical, a toxic metabolite (Galelli and Castro, 1998), which attacks the cellular components and produces oxidative injury (Lee et al., 2003).

A different scenario was observed when the TBARS level was measured in the plasma. There was no significant increase in oxidative stress 24 h after exposure to CCl_4. It is known that orally administered CCl_4 has a half-life of 4 to 5 h (Larson and Plaa, 1996)

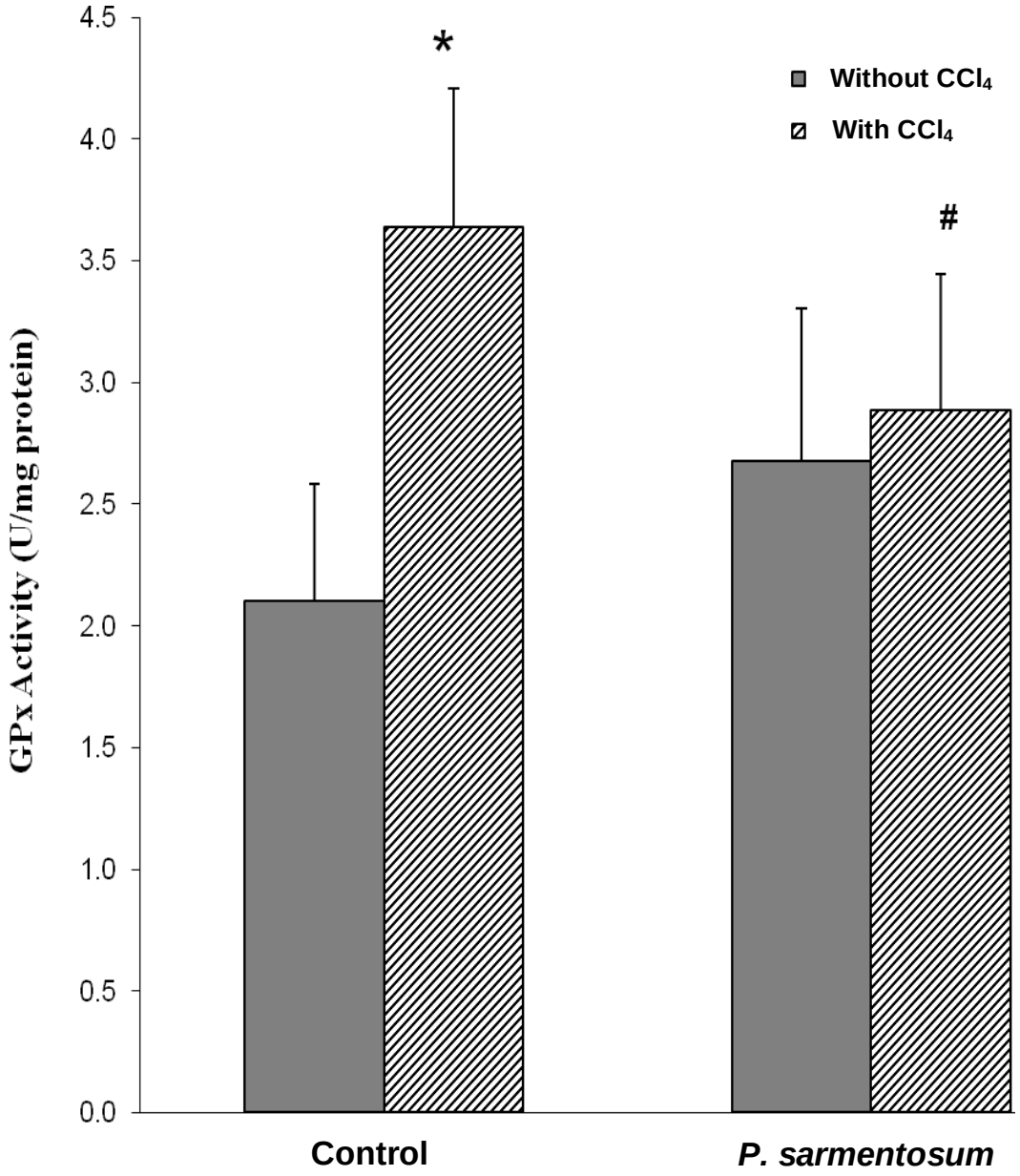

Figure 3. The effect of oral *Piper sarmentosum* supplementation (125 mg/kg body weight, 28 days) on CCl_4-induced (1 ml/kg body weight) change in lung glutathione peroxidase (GPx) activity. Bars represent mean ± SEM (n = 8). *Significantly different at $p<0.05$ compared to non-exposed control. #Significantly different at $p<0.05$ compared to the exposed control.

that after 24 h, the level of CCl_4 inducing oxidative stress changes in the plasma had diminished. We also found that, with the insignificant increase in TBARS, the effect of *P. sarmentosum* remained unchanged in CCl_4 group as compared to the treated control.

The important antioxidant enzymes in the lungs include glutathione peroxidase and superoxide dismutase. These enzymes are known to protect the lungs against oxidative damages. Superoxide dismutase protects cell by converting superoxide anions ($O_2^{\bullet-}$) to H_2O_2. These molecules, which are toxic to cells, can be further broken down to release hydroxyl radical ($^{\bullet}OH$), a reactive species which is more damaging to the cells. The enzymes responsible for detoxifying H_2O_2 are glutathione peroxidase and catalase, which prevents the formation of $^{\bullet}OH$ by converting H_2O_2 to non-harmful products, which are oxygen and water (Jung and Henke, 1996; Michiels et al., 1994).

In this study, a significant increase in the lung glutathione peroxidase enzyme activity was observed in

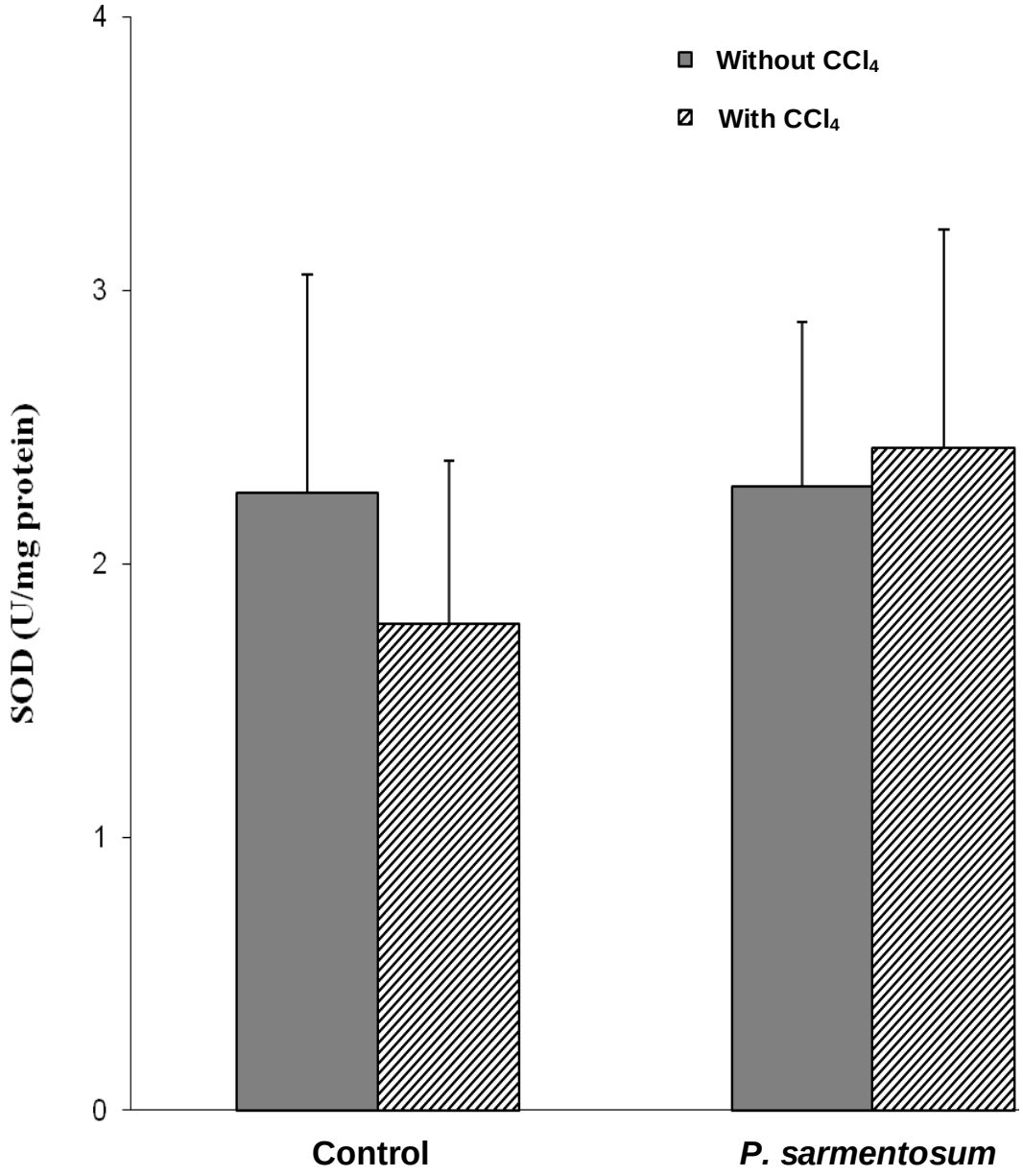

Figure 4. Lung superoxide dismutase (SOD) activity in *Piper sarmentosum*-supplemented rats (125 mg/kg body weight, orally) for 4 weeks following oral administration of CCl_4 (1 ml/kg body weight). Bars represent mean ± SEM of 8 animals. No differences were between groups (P>0.05).

rats exposed to CCl_4 in comparison to the non-exposed control. Other studies had shown that this endogenous enzyme is important in preventing damages in the lung tissue (Smith et al., 1997; De Raeve et al., 1997), where it had been shown to detoxify reactive oxygen species in the lungs (Comhair and Erzurum, 2002). The increased level of glutathione peroxidase can be explained by the findings reported by Comhair and Erzurum (2002), where the gene expression of the enzyme was increased in the bronchiole epithelial cells and epithelial lining fluid when exposed to oxidative stress. The increase of this enzyme only occurred 24 h post exposure to the oxidant.

Interestingly we found that the supplementation with *P. sarmentosum* for 28 days were able to maintain the glutathione peroxidase level towards the normal value. It is possible that the rats which received *P. sarmentosum* supplementation were already protected from the oxidant damages in the lung as shown by the normal TBARS levels in this group after exposure to CCl_4. Thus, an increase in the glutathione peroxidase enzyme which is known to be regulated by an increase in gene expression as mentioned earlier (Comhair and Erzurum, 2002) did

not occur with any oxidative stress occurrence in these rats.

Tirkey et al. (2005) showed that CCl_4 reduced the important endogenous enzymes activities in the body including superoxide dismutase. In our study, although the superoxide dismutase enzyme activity in the lung of rats exposed to CCl_4 was not significantly different to the non-exposed control, however, a trend of reduction in this enzyme activity was observed. To the best of our knowledge, there is no other study reporting the detrimental effects of CCl_4 on antioxidant enzymes in the lungs. Other studies had demonstrated decreased antioxidant enzymes (superoxide dismutase, glutathione peroxidase and catalase) activities in kidneys, heart, brain (Khan et al., 2009; Jayakumar et al., 2008) as well as the liver (Sreelatha et al., 2009) following CCl_4 intoxication.

In conclusion, *P. sarmentosum* supplementation at 125 mg/kg/day for 28 days was able to decrease oxidative stress in lungs induced by a single dose of CCl_4 by reducing the lung TBARS content and maintaining the glutathione peroxidase activity towards the normal level. These findings suggest that methanolic extract of *P. sarmentosum* possesses good antioxidant property which should be explored further.

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