EFFECTS OF METHANOLIC LEAF EXTRACT OF *PTEROCARPUS SANTALINOIDES* DC ON BLOOD OXIDATIVE STRESS AND ANTI-OXIDANT PARAMETERS OF ALBINO RATS GIVEN TOXIC DOSES OF CARBON TETRACHLORIDE

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Abstract
This study investigated the effects of methanolic leaf extract of *Pterocarpus santalinoides* DC on blood oxidative stress and anti-oxidant parameters of albino rats given toxic doses of carbon tetrachloride (CCl_4). Fresh leaves of *P. santalinoides* were collected, dried under shade, ground into powder, and extracted by cold maceration using 80% methanol. The extract obtained was filtered, concentrated to dryness and referred to as *Pterocarpus santalinoides* methanolic leaf extract (PSMLE). Thirty male albino rats (*Rattus norvegicus*) of 12 weeks of age were randomly assigned into 6 groups (A – F) of 5 rats each. A mixture of 1 ml/kg CCl_4 in equal volume of olive oil (50% v/v) was injected intraperitoneally to groups A – E at 3 days interval for 12 days (days 0, 3, 6, 9 and 12) to induce sub-acute toxicity. Group A was treated with 10 ml/kg distilled water as placebo and served as negative control (induced untreated), groups B, C and D were treated with 50, 250 and 500 mg/kg of PSMLE respectively. Group E was treated with 100 mg/kg Silymarin as positive control, while group F was treated with 10 ml/kg distilled water as placebo (normal control not given CCl_4). Treatment was given *per os* twice daily for 15 days. Blood was collected on day 15 post-initiation of treatment for determination of malondialdehyde (MDA), catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and total plasma glutathione activity levels. Treatment with PSMLE at 250 and 500 mg/kg especially led to significantly lower ($P < 0.05$) blood MDA levels, and significantly higher ($p < 0.05$) activity concentrations of catalase, SOD, GPx and total glutathione in the treated rats when compared to the untreated negative control. It was concluded that treatment with 250 and 500 mg/kg PSMLE significantly ameliorated CCl_4-induced oxidative stress and enhanced blood levels of antioxidant parameters, which suggests that the extract may be useful in diseases mediated by and associated with oxidative stress.

Key words: Oxidative stress, Anti-oxidants, *Pterocarpus santalinoides,* Methanolic leaf extract, Carbon tetrachloride.

Introduction

Oxidative stress is a state in which oxidation exceeds the anti-oxidant systems in the body leading to imbalance between the generation of reactive oxygen species (ROS) and the level of anti-oxidants in the biological system (Yoshikawa and Naito, 2002). Oxidative stress occurs when free radicals which are not neutralized by antioxidants go on to create more volatile free radicals and damage cell membranes, vessels, proteins, fats and DNA. Biological free radicals are highly unstable reactive molecules that have electrons available to react with various organ substrates such as DNA, proteins and lipids. Oxidative stress is known to be involved in the pathogenesis of lifestyle-related diseases including atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, liver diseases and malignancies (Yoshikawa and Naito, 2002). Carbon tetrachloride (CCl_4) is a commonly used model chemical for the induction of oxidative stress-mediated hepatotoxicity (Boll *et al*., 2001; Weber *et al.*, 2003; Kim *et al*., 2010).

Anti-oxidants are compounds that prevent chemical damage caused by free radicals. Many of the natural antioxidants such as flavonoids and glycosides are very important in the prevention of diseases associated with oxidative stress (Yi-Fang *et al*., 2002; Aruoma, 2003). Plants are rich sources of natural antioxidants that can protect against oxidative stress and thus play important role in the chemoprevention of diseases

that have their etiology and pathophysiology in ROS (Ames *et al*., 1993; Atawodi, 2005). There has been an increase in interest in the therapeutic potential of plants as antioxidants in reducing free radical-induced tissue injury (Schuler, 1990). Many plant species have been investigated in the search for novel antioxidants (Chu, 2000; Mantle *et al*., 2000; Koleva *et al*., 2002; Oke and Hamburger, 2002), but generally there is still a need to investigate the anti-oxidant potential of some other plant species.

Pterocarpus santalinoides DC is an indigenous Nigerian plant in the family *Papilionaceae*. It is commonly known as "red sandal wood" in English and "nturukpa" in Igbo language (Adetunji, 2007; Anowi *et al*., 2012). The leaves are used in folk medicine for the treatment of various ailments such as heart and liver diseases (Adesina, 1982; Okwu and Ekeke, 2003). Phytochemical analysis of the leaf extracts of *P. santalinoides* have shown the presence of tannins, alkaloids, flavonoids, saponins, carbohydrates, glycosides, phenols, terpenes and sterols (Anowi et al., 2012; Eze et al., 2012; Ihedioha et al, 2017; Ihedioha et al., 2018). Previous studies have suggested that the hepato-protective effects of methanol leaf extract of *P. santalinoides* in acetaminophen–induced hepatotoxicity may be due to its anti-oxidant phytochemical composition (Ihedioha et al., 2017). Also, earlier studies that showed that aqueous leaf infusion of *P. santalinoides* had lipid lowering effects (Ihedioha et al., 2018) hinted that this capability may be related to the extract's anti-oxidant capacity. Based on the various medicinal uses of *P. santalinoides* especially in the treatment of diseases in which oxidative stress is known to play a role, the purpose of the present study was to evaluate the effects of methanolic leaf extract of *Pterocarpus santalinoides* DC on blood oxidative stress and anti-oxidant parameters of albino rats given toxic doses of carbon tetrachloride (CCl_4).

Materials and Methods

Fresh leaves of *Pterocarpus santalinoides* used for the study were collected in November 2016, and identified by a plant taxonomist at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The leaves were dried under shade and pulverized. Five hundred grammes (500 g) of the ground leaves were extracted with 80% methanol using the cold maceration extraction technique. The resulting extract was concentrated to dryness with a rotary evaporator (Buchi, Switzerland), and referred to as *Pterocarpus santalinoides* methanol leaf extract (PSMLE).

A total of thirty (30) male albino rats (*Rattus norvegicus*) of 12 weeks of age were used for the study. The rats were randomly assigned to 6 groups A – F of five (5) rats per group. Sub-acute toxicity was induced in rat groups A – E using CCl_4 model (Singh et al., 2012). A mixture of 1 ml/kg CCl_4 in equal volume of olive oil (50% v/v) was injected intraperitoneally to groups A – E at 3 days interval (days 0, 3, 6, 9 and 12) for 12 days to induce sub-acute toxicity. Group A served as negative control (induced untreated), groups B, C and D were treated *per os* twice daily with 50, 250 and 500 mg/kg of PSMLE respectively. Group E was treated with 100 mg/kg silymarin (a standard hepatoprotective drug) p*er os* twice daily as positive control, while group F was treated *per os* twice daily with 10 ml/kg distilled water as placebo (normal control not given CCl_4). Treatment was done for 15 days, and the albino rats used for the study were cared for and handled humanely all through the study. Blood was collected on day 15 post-initiation of treatment for evaluation of blood levels of oxidative stress and anti-oxidant parameters, using the following standard methods: malondialdehyde (MDA) – modified thiobarbituric acid method (Draper & Hadley, 1990); total glutathione – modified enzyme recycling method (Tipple & Rogers, 2012); catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity – methods described by Weydert & Cullen (2010).

Data obtained from the study were subjected to one way analysis of variance (ANOVA). Variant means were separated post-hoc using the least significant difference method. Significance was accepted at $p < 0.05$, and a summary of the results were presented as means with standard error.

Results and Discussion

Results of the MDA assay showed a dose-dependent variation in MDA levels across the groups, with group A being significantly higher ($p < 0.05$) than groups C, D, E and F, but not significantly different ($p > 0.05$) from group B (Table 1). This implied that there was higher free radical production in groups A and B when

compared to other groups, as an increase in free radicals causes increased production of MDA (DelRio et al., 2005). MDA is one of the final products of polyunsaturated fatty acids peroxidation in cells and is a known marker of oxidative stress status in several diseases (Gawel et al., 2004). Catalase activity concentration of group A rats was significantly lower ($p < 0.05$) than that of groups B, C, D, E and F rats, and there were no significant differences ($p > 0.05$) in catalase activity of groups B, C, D and E (Table 1). This is an indication that group A rats had no protection from oxidative damage as compared to other groups. Catalase is a very important enzyme in protecting the cell from oxidative damage (Chelikani et al., 2004). It is the main enzyme that removes hydrogen peroxide a reactive oxygen species and blocks oxidative stress (Gaetani et al., 1996). Results of the SOD assay also showed significantly lower ($p < 0.05$) superoxide dismutase (SOD) level of groups A and B rats when compared to other groups (Table 1). This is also an indication that groups A and B rats had no significant protection from oxidative damage as compared to other groups. Superoxide dismutase is an important antioxidant defense in living cells exposed to oxygen. Earlier studies had shown that treatment with SOD decreases reactive oxygen species generation and oxidative stress (Fridovich, 1997; Gongora et al., 2006). Glutathione peroxidase (GPx) levels followed the same pattern as catalase and SOD with groups A and B being significantly lower ($p < 0.05$) than groups C, D, E and F (Table 1). The main biological role of GPx is to protect the organism from oxidative damage. Its biochemical function is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. Blood levels of GPx have been reported to be low in some important diseases, and had significantly improved when anti-oxidants were administered (Lubos et al., 2011). Results of the total glutathione assay showed a dose dependent variation in plasma total glutathione level across the groups, with group A rats being significantly lower ($p < 0.05$) than those of other groups, and group F being significantly higher ($p < 0.05$) than other groups (Table 1). Plasma total glutathione is one of the major endogenous antioxidants produced by cells which participates directly in the neutralization of free radicals and reactive oxygen species, as well as maintaining exogenous antioxidants such as vitamins C and E in their reduced (active) forms (Dringen, 2000). The higher total glutathione level recorded in this study for groups B, C, and D implies a dose-dependent increase in anti-oxidant activity in these groups.

Table 1. The blood oxidative stress and anti-oxidant parameters of rat groups* given CCl_4 and treated with varied doses of *Pterocarpus santalinoides* methanolic leaf extract (PSMLE).

Oxidative stress and antioxidant parameters	Means, with standard deviation in brackets					
	Group A	Group B	Group C	Group D	Group E	Group F
Malondialdehyde (µmol/L)	17.61 ^{a} (3.32)	14.19 ^{ab} (4.29)	9.65 ^{bc} (2.87)	7.52 ^{cd} (3.30)	7.65 ^{cd} (2.88)	4.05 ^{d} (1.90)
Catalase (IU/ml)	12.18 ^{a} (2.36)	21.55 ^{b} (2.38)	20.92 ^{b} (3.35)	20.35 ^{b} (3.49)	23.14 ^{bc} (4.40)	29.76 ^{c} (7.99)
Superoxide dismutase (IU/ml)	0.221 ^{a} (0.020)	0.257 ^{ab} (0.049)	0.300 ^{c} (0.024)	0.278 ^{bc} (0.020)	0.294 ^{bc} (0.027)	0.299 ^{c} (0.019)
Glutathione peroxidase (IU/L)	82.50 ^{a} (10.74)	88.41 ^{a} (12.89)	119.35 ^{b} (20.17)	124.29 ^{b} (23.90)	127.16 ^{b} (17.27)	190.25 ^{c} (18.58)
Total Glutathione (µg/µl)	0.016 ^{a} (0.006)	0.029 ^{b} (0.004)	0.039 ^{bc} (0.005)	0.041 ^{bc} (0.005)	0.046 ^{c} (0.006)	0.068 ^{d} (0.015)

^{a,b,c} Different alphabetical superscripts in a row indicate significant differences ($p < 0.05$) between the groups.

* Groups: Group A – CCl_4 and no treatment; Group B – CCl_4 + 50 mg/kg bw PSMLE; Group C – CCl_4 + 250 mg/kg bw PSMLE; Group D – CCl_4 + 500 mg/kg bw PSMLE; Group E – CCl_4 + 100 mg/kg bw Silymarin; Group F – No CCl_4 and no treatment.

Conclusion

Based on the results of the study, it was concluded that treatment of oxidative stress mediated CCl_4-induced toxic injury in albino rats with methanolic leaf extract of *P. santalinoides* at the doses of 250 and 500 mg/kg, especially led to significantly lower ($P < 0.05$) blood MDA levels, and significantly higher ($p < 0.05$) activity concentrations of catalase, SOD, GPx and total glutathione of the treated rats when compared to the untreated negative control. The implication of these findings is that extracts of the leaves of *P. santalinoides* may be beneficial in the treatment and management of diseases mediated by or associated with oxidative stress.

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