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HEPATOPROTECTION BY *Pterocarpus santalinoides* METHANOL LEAF EXTRACT DURING CARBON TETRACHLORIDE INDUCED SUBACUTE HEPATOTOXICITY: EFFECTS ON THIOPENTONE-INDUCED SLEEP DURATION IN ALBINO RATS

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ABSTRACT

This study investigated the effects of hepatoprotection by Pterocarpus santalinoides methanol leaf extract during carbon tetrachloride-induced sub-acute hepatotoxicity on thiopentone sleeping time in albino rats. Leaves of Pterocarpus santalinoides were dried under shade, pulverized into powdered form, extracted by cold maceration with 80% methanol, and used for the study. Thirty adult male albino rats randomly assigned to six groups (A – F) of five rats each were used for the study. Carbon tetrachloride was administered to groups A – E rats at the dose of 1 ml/kg intraperitoneally at three-day intervals from day 0 to day 12 of the experiment (total of five times). Group A rats were treated with 10 ml/kg distilled water (negative control), groups B, C and D rats were treated with 50, 250 and 500 mg/kg extract, respectively, Group E rats were treated with 100 mg/kg silymarin as positive control, while Group F rats were given only 10 ml/kg distilled water (normal control). Treatment with the extract, silymarin and distilled water placebo were done per os, twice daily, from day 1 (a day after the first carbon tetrachloride injection) up to day 15 (end of the study). Blood samples were collected from all the rats on day 15 for liver function tests. After the blood sample collection, a single intra-peritoneal injection of 50 mg/kg sodium thiopentone was given to all the rats to induce narcosis. The duration of sleep (sleeping time) for each rat in all the groups was recorded. Results showed that the group D rats given 500 mg/kg extract had significantly ($p < 0.05$) shorter sleep duration when compared to the negative control (Group A) and the group treated with 50 mg/kg extract (Group B). Also, co-treatment at the dose of 250 and 500 mg/kg extract and 100 mg/kg silymarin (Group E) significantly ($p < 0.05$) protected hepatocellular integrity and ameliorated impaired hepatic excretory function induced by carbon tetrachloride. It was concluded that the extract at 500 mg/kg dose significantly

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shortened sodium thiopentone sleeping time in albino rats with hepatopathy caused by carbon tetrachloride.

Keywords: *Pterocarpus santalinoides* leaf extract, CCl_4-induced hepatotoxicity, Sodium thiopentone-induced sleeping time, Rats.

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INTRODUCTION

Barbiturates are narcotic drugs which can reduce anxiety and produce a calming effect by inducing the onset of sleep as well as maintaining sleeping duration [1,2]. They are extensively used in the treatment of different psychiatric disorders including anxiety and insomnia. Sodium thiopentone (thiopental, pentothal) is the thiobarbiturate analog of pentobarbital and an analog of thiobarbital [3,4]. It is a rapid-onset, ultra-short-acting general anesthetic, which rapidly reaches the brain to cause unconsciousness within 30–45 seconds after intravenous administration. As with all lipid-soluble anaesthetic drugs, the short duration of action of sodium thiopentone is due to its redistribution away from central circulation towards muscle and fat tissue due to its very high fat–water partition coefficient [4]. The free fraction in the blood is metabolized in the liver to pentobarbital 5-ethyl-5-(1'-methyl-3'-hydroxybutyl)-2-thiobarbituric acid, and 5-ethyl-5-(1'-methyl-3'-carboxypropyl)-2-thiobarbituric acid [4,5], but the ability of the liver to transform the drug to these inactive forms may be impaired by hepatopathy from extraneous toxic injuries; and such incidence prolongs the drug-induced narcosis or sleeping time.

Carbon tetrachloride (CCl_4), a hepatotoxicant is metabolized to trichloromethyl radical (CCl_3O-), and trichloromethylperoxy (CCl_3OO^-) radical, which impair hepatocellular processes by oxidative injuries causing hepatopathy and inhibiting microsomal enzymes necessary for barbiturate metabolism [6,7,8].
Pterocarpus santalinoides DC (red sandal wood) is a tree with bright yellow flowers and irregularly shaped fruit pods [9,10]. In Southeastern Nigeria, the tender leaves of *P. santalinoides* are used as vegetable in soups, and may be helpful traditionally in treating heart and liver diseases, diabetes mellitus, arthritis, nausea and vomiting, infertility and stomach ache [11,12,13,14]. *Pterocarpus santalinoides* leaf extract is reported to be protective against toxic injuries to the liver [15,16,17,18,19,20,21,22], but there has been no report on the effect of *P. santalinoides* methanol leaf extract on sodium thiopentone-induced sleeping time (narcosis) in individuals exposed to hepatotoxic xenobiotics. The aim of this study therefore was to evaluate the effects of hepatoprotection by *P. santalinoides* methanol leaf extract during CCl_4 - induced sub-acute hepatotoxicity on thiopentone-induced sleeping time in albino rats.

MATERIALS AND METHODS

The plant leaves used for the study were collected from *Pterocarpus santalinoides* tree at Nru Community in Nsukka Local Government Area of Enugu State, Nigeria. The plant was identified and authenticated by a plant taxonomist at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. A Voucher Specimen (UNH No. 2017/02) was deposited in the University of Nigeria, Nsukka Herbarium. The leaves were allowed to dry under shade and later finely ground into powder using a grinding machine. Extraction was done with 80% methanol (Sigma-Aldrich, St. Louis, Missouri, USA) by the cold maceration technique, with intermittent shaking at two-hour intervals for 48 hours. The extract (PME) obtained was filtered with Whatman size 1 filter paper and concentrated to dryness in a rotary evaporator (Buchi, Switzerland).

Thirty adult male albino rats (*Rattus norvegicus*), weighing between 180 g – 200 g were procured from the Laboratory Animal Unit of the University of Nigeria, Nsukka. They were housed in stainless steel cages in a fly proof animal house at room temperature and allowed 2 weeks to acclimatize before the commencement of the study. They were fed commercial pelletized grower's feed (Grand Cereals Ltd, Jos, Nigeria) and provided with clean drinking water *ad libitum*. The study was approved by the Institutional

Animal Care and Use Committee of the University of Nigeria, Nsukka (FVM-UNN-IACUC/2016/0814). Guidelines for the humane handling of animals were followed all through the study.

The rats were randomly assigned to six groups (A – F) of five rats per group. Rats in groups A – E were given intra-peritoneal injections of 1 ml/kg CCl_4 (Sigma-Aldrich, St. Louis, Missouri, USA) in equal volume of olive oil at the beginning of the study (day 0) and afterwards at three-day intervals for 12 days (days 0, 3, 6, 9 and 12) [20,23,24]. Additionally, Group A rats were given distilled water at the dose of 10 ml/kg *per os* twice daily as placebo and served as negative control, while rats in Groups B, C and D were treated *per os* twice daily with 50, 250 and 500 mg/kg of PME, respectively. Group E rats were treated with silymarin (Sigma-Aldrich, St. Louis, Missouri, USA), a hepatoprotective drug, at the dose of 100 mg/kg *per os* twice daily, and the silymarin (SLM)-treated group served as positive control, while Group F rats were given distilled water at the dose of 10 ml/kg *per os* twice daily as placebo, and served as normal control (not given CCl_4). Treatments with PME, silymarin and the distilled water placebo commenced on day 1 and were continued for 15 days.

Blood samples (2 ml from each rat) were collected without anticoagulant by the orbital technique [25] on day 15 (after the last treatment), to harvest serum for evaluation of levels of enzyme markers of hepatocellular damage [alanine aminotransferase (ALT), aspartate aminotranferase (AST), and alkaline phosphatase (ALP) activities] and liver function (serum albumin, total cholesterol and total bilirubin), following standard biochemical procedures, using test kits [Quimica Clinica Aplicada (QCA), Spain]. Serum ALT and AST activities were assayed following the Reitman-Frankel method [26, 27], while the serum ALP activity was determined by the phenolphthalein monophosphate method [27,28]. Serum albumin levels were determined based on the bromocresol green method [29], serum total cholesterol by the enzymatic colorimetric method [30], and serum total bilirubin by the Jendrassik-Grof method [31].

After blood sample collection on day 15, a single intra-peritoneal injection of 50 mg/kg sodium thiopentone (Vitapure Corp., Mumbai, India) was administered to each rat to induce narcosis and determine onset and duration of sleep in each rat.

Data analysis

Data obtained from the study were analyzed using one way analysis of variance (ANOVA) and variant means were separated post hoc using the least significant difference (LSD) method. Significance was accepted at $P < 0.05$. The statistical package of the social sciences (SPSS) software was used for the analysis. Results are presented as means ± SEM.

RESULTS

There was prolongation of sleep duration (narcosis) in all the hepatopathic rat (HPR) groups (A-E) treated with CCl_4, with Group A (negative control) having the longest duration of sleep (Figure 1). Treatment with PME at all the doses led to significant ($p < 0.05$) shortening of the sleep duration in a dose-dependent manner, with Group D (500 mg/kg PME) having the shortest duration of sleep among the PME treated HPR groups. The mean sleep duration of Group D (500 mg/kg PME) rats was significantly ($p < 0.05$) shorter than those of Groups A (negative control) and B (50 mg/kg PME) rats, but was comparable ($p > 0.05$) to the sleep duration of the Group C (250 mg/kg PME) and Group E treated with SLM. The sleep duration of all the HPR groups were however significantly longer ($p < 0.05$) than that of the normal control (Group F).

Treatment with PME at the doses of 250 and 500 mg/kg in HPR groups (C and D) and treatment of HPR group with SLM (Group E) led to significantly ($p < 0.05$) lower serum ALT activity when compared to the HPR group A (negative control without hepatoprotective treatment) (Table 1). Treatment with 250 mg/kg PME (Group C) and 100 mg/kg SLM (Group E) led to significantly lower ($p < 0.05$) serum AST activity, which was comparable ($p > 0.05$) to the serum AST activity of the normal control (Group F).

There were no significant ($p > 0.05$) differences in serum ALP activity between PME-treated HPR groups (B, C and D), although there was lowering of serum ALP activity which occurred in a dose dependent manner. The serum ALP activity of the normal control (Group F) was significantly ($p < 0.05$) lower than that of the negative control (Group A) and all the PME-treated groups.

Table 2 shows the serum levels of albumin, cholesterol and bilirubin in rat groups co-treated with graded doses of PME and hepatotoxic dose of CCl_4. There were no significant ($p > 0.05$) variations between the negative control (Group A) and the PME-treated groups (B, C and D) in their serum albumin levels (Table 2). However, treatment of HPR group with SLM (Group E) led to significantly ($p < 0.05$) higher serum albumin level that compared ($p > 0.05$) with that of the normal control (Group F). Treatment with 500 mg/kg PME (Group D) led to significantly ($p < 0.05$) lower serum total cholesterol level when compared to the negative control (Group A), and there were no significant ($p > 0.05$) differences in serum total cholesterol levels between Group D and Groups B, C, E and F. Treatment with 250 mg/kg PME (Group C), 500 mg/kg PME (Group D) and 100 mg/kg SLM (Group E) in HPR groups led to significantly ($p < 0.05$) lower serum total bilirubin level when compared to the negative control (Group A) and HPR group treated with 50 mg/kg PME (Group B).

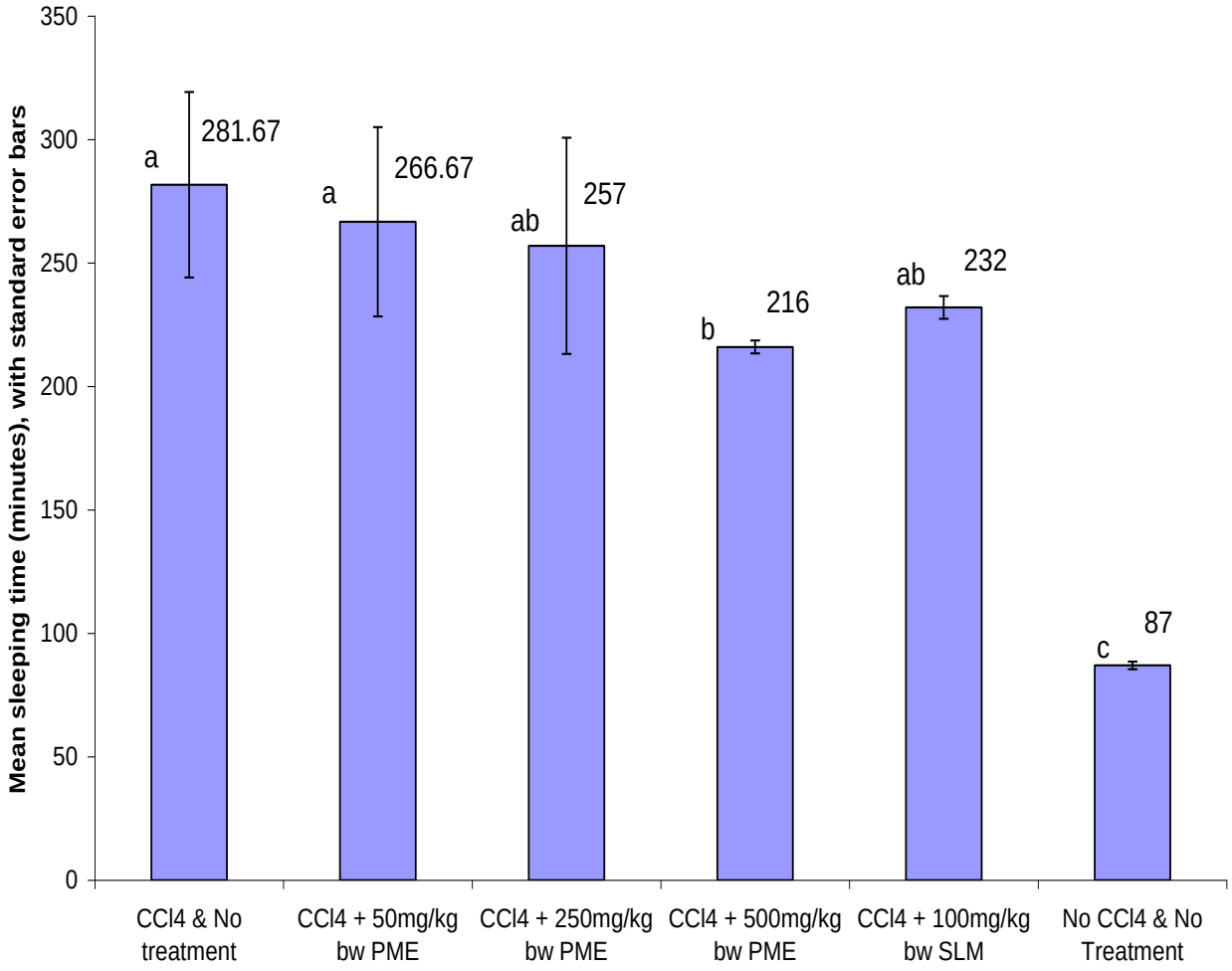

Figure 1. The sleep duration of rat groups co-treated with varied doses of *Pterocarpus santalinoides* methanol leaf extract (PME) and hepatotoxic dose of carbon tetrachloride (CCl_4), and then given 50 mg/kg body weight of sodium thiopentone.

Table 1. The serum enzyme activity of rat groups co-treated with graded doses of *Pterocarpus santalinoides* methanol leaf extract (PME) and hepatotoxic dose of carbon tetrachloride (CCl_4), and then given 50 mg/kg body weight of sodium thiopentone.

*Groups	Mean ± standard error (IU/L)		
	Alanine aminotransferase	Aspartate aminotransferase	Alkaline phosphatase
Group A	103.2 ± 4.64 [a]	123.21 ± 7.84 [a]	389.05 ± 59.72 [a]
Group B	99.45 ± 5.93 [a]	120.04 ± 7.39 [a]	391.12 ± 45.32 [a]
Group C	47.58 ± 6.91 [b]	76.98 ± 7.74 [b]	359.75 ± 10.11 [a]
Group D	76.83 ± 6.21 [c]	105.87 ± 5.21 [a]	339.02 ± 17.88 [a]
Group E	46.87 ± 7.24 [b]	75.16 ± 6.63 [b]	278.34 ± 21.57 [ab]
Group F	30.66 ± 4.83 [b]	63.12 ± 6.38 [b]	105.73 ± 20.34 [b]

[a, b, c] Different alphabetical superscripts in a column indicate significant ($p < 0.05$) differences between the groups; *Groups: Group A – CCl_4 + 10 ml/kg distilled water (negative control); Group B – CCl_4 + 50 mg/kg PME; Group C – CCl_4 + 250 mg/kg PME; Group D – CCl_4 + 500 mg/kg PME; Group E – CCl_4 + 100 mg/kg SLM (positive); Group F – No CCl_4, no treatment (normal control)

Table 2. The serum levels of albumin, cholesterol and bilirubin in rat groups co-treated with graded doses of *Pterocarpus santalinoides* methanol leaf extract (PME) and hepatotoxic dose of carbon tetrachloride (CCl_4), and then given 50 mg/kg body weight of sodium thiopentone.

*Groups	Mean ± standard error (mg/dl)		
	Serum Albumin	Serum Total Cholesterol	Serum Total Bilirubin
Group A	3.45 ± 0.23 [a]	82.12 ± 8.11 [a]	0.71 ± 0.05 [a]
Group B	3.43 ± 0.16 [a]	62.97 ± 9.93 [ab]	0.64 ± 0.13 [a]
Group C	3.48 ± 0.15 [a]	61.65 ± 3.05 [ab]	0.36 ± 0.08 [b]
Group D	3.46 ± 0.10 [a]	55.73 ± 2.39 [b]	0.40 ± 0.04 [b]
Group E	3.69 ± 0.12 [b]	63.15 ± 5.54 [ab]	0.37 ± 0.05 [b]
Group F	3.73 ± 0.11 [b]	62.45 ± 3.56 [ab]	0.38 ± 0.04 [b]

[a, b, c] Different alphabetical superscripts in a column indicate significant ($p < 0.05$) differences between the groups

*Groups: Group A – CCl_4 + 10 ml/kg distilled water (negative control); Group B – CCl_4 + 50 mg/kg PME; Group C – CCl_4 + 250 mg/kg PME; Group D – CCl_4 + 500 mg/kg PME; Group E – CCl_4 + 100 mg/kg SLM (positive); Group F – No CCl_4, no treatment (normal control)

DISCUSSION

The prolonged sleep duration in HPR groups given thiopentone injections was due to the liver damage caused by CCl_4 toxicity. Thiopentone is catabolized by the microsomal enzymes in hepatocytes [4,5], leading to cessation of its action. Toxic hepatopathy causes depletion of the microsomal enzymes leading to the accumulation of thiopentone in the blood and prolongation of its narcotic action. According to earlier reports [8], catabolic inactivation is important in determining the duration of the drug action. The finding in this study that the HPR group treated with 500 mg/kg PME had significantly shorter sleep

duration when compared to other HPR groups, suggests that PME at this dose may have preserved the activity of the microsomal enzymes of the hepatocytes.

The findings that PME at the doses of 250 and 500 mg/kg led to significant lowering of the CCl_4-induced elevation of serum ALT, AST, serum total cholesterol and/or serum bilirubin, imply that PME at these doses was protective of the hepatocellular integrity and hepatic excretory function. This hepatoprotective activity of PME compared with that of silymarin (a standard hepatoprotective drug), and concurs with earlier reports [18,20,32], which indicated significant lowering of serum ALT, AST and bilirubin of rat groups treated with *P. santalinoides* leaf extracts.

The shortening of sleep duration was highest in the group treated with 500 mg/kg PME (Group D), while hepatocellular integrity and hepatic excretory function was most protected at the dose of 250 mg/kg PME (Group C). This implies that the facilitation of microsomal enzyme activation and thus thiopentone catabolism is not directly related to hepatocellular integrity and hepatic excretory function. This confirms the commonly known multiple unrelated functions of the liver and the varied effects of liver disorders/diseases on drug pharmacokinetics [33,34,35].

It was concluded that the administration of PME (500 mg/kg) significantly shortened thiopentone-induced sleep duration prolonged by toxic damage to the liver from CCl_4 intoxication of rats because of the hepatoprotective effect of the extract.

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