JOURNAL OF VETERINARY AND APPLIED SCIENCES
VOLUME 14, ISSUE 2: 828 – 841 (2024)
Published by: Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria
ISSN: 2315-6856; e-ISSN: 2636-5553; Website: www.jvasonline.com

Comparison of the hepatotherapeutic efficacy of orally and parenterally administered methanol extract of leaves of *Pterocarpus santalinoides* on carbon tetrachloride-induced liver damage in albino rats

Thelma E. Ihedioha [1] *, John I. Ihedioha [2] and Miriam Keswet [1]

[1] Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

[2] Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

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Abstract

The occurrence of liver diseases is on the increase and there are limited medicines that can be administered through various routes for its treatment. Orally administered methanol extract of the leaves of *Pterocarpus santalinoides* (MELPS) has been reported to be hepatotherapeutic, but administration via other routes has not been investigated. This study compared the hepatotherapeutic efficacy of orally and parenterally administered MELPS on carbon tetrachloride (CCl_4)-induced liver damage in albino rats. The extract was prepared by cold maceration of the dried leaves. Thirty adult female albino rats randomly assigned into six groups A – F of five rats each were used for the study. Liver damage was induced in Groups A – E using CCl_4 administered at three day intervals for 12 days. Group A was treated with 10 ml/kg distilled water placebo *per os* as negative control. Groups B, C and D were treated with 250 mg/kg MELPS, administered orally, intramuscularly (IM) and intraperitoneally (IP), respectively. Group E was treated with 200 mg/kg Silymarin *per os* as positive control, while Group F was given 10 ml/kg distilled water *per os* as normal control. Treatment commenced on day 12 (after liver damage has been induced), and was done twice daily for five days. Blood samples were collected on days 0, 12 and 16 for serum biochemistry assay. The rats were humanely sacrificed on day 16, the livers were carefully eviscerated and weighed, and relative liver weights (RLW) calculated. Results showed that the CCl_4 administration resulted to significantly ($p < 0.05$) higher serum ALT and AST activities in Groups A, B, C, D and E when compared to Group F on day 12. Treatment with 250 mg/kg MELPS via oral, IM and IP routes, and 200 mg/kg silymarin (Groups B, C, D and E) led to significantly lower ($p < 0.05$) serum ALT and AST activities, lower liver weights and RLW on day 16 when compared to Group A. Groups B, C and D also had significantly ($p < 0.05$) higher serum ALP activity and significantly ($p < 0.05$) lower serum cholesterol levels when compared to Groups A and E on day 16. It was concluded that both IM and IP routes of administration were efficacious and compared effectively with the oral route of administration. Thus, methanol extract of the leaves of *Pterocarpus santalinoides* can be effectively administered parenterally as a hepatotherapeutic agent.

Keywords: *Pterocarpus santalinoides;* Leaf extract; Hepatotherapy; Oral route; Intramuscular route; Intraperitoneal route.

*** Correspondence:** Thelma E. Ihedioha; Email: thelma.ihedioha@unn.edu.ng; Phone: +2348036868258

Article History: Initial manuscript submission received – Sept. 14, 2024; Final revised form received – Dec. 07, 2024; Accepted for publication – Dec. 19, 2024; Published – Dec. 30, 2024.

Introduction

The liver is one of the major vital organs responsible for several key metabolic processes in the body (Asrani *et al*, 2019). It is responsible for the detoxification of drugs and xenobiotics; thus, it is constantly exposed to xenobiotics which may cause liver damage/hepatotoxicity (Saukkonen *et al*., 2006). Liver damage is a major health problem; the manifestations of which are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant liver failure (Saukkonen *et al*., 2006). Toxic liver diseases are amongst the most common causes of morbidity and mortality in animals and humans (Asrani *et al*., 2019), and it is reported to account for approximately two million human deaths per year worldwide (Burroughs and McNamara, 2003). The occurrence of liver diseases/liver failure is on the increase and there are limited available medications for its treatment/management. Most of the effective remedies are administered orally, limiting their use in some animals, such as ruminants; some are also expensive and not easily available. Therefore, the search for alternative therapies that can be administered via other routes apart from the oral route, and which can be readily available at low cost is of paramount importance.

Medicinal plants constitute a large part of traditional medicines and continue to provide mankind and animals with therapeutic remedies and novel drug leads (Gurib-Fakim, 2006; Newman and Cragg, 2012). *Pterocarpus santalinoides* DC is an indigenous Nigerian plant in the family *Papilionaceae* (*Fabaceae*), which occurs throughout the tropics (Ogan, 2004, Anowi *et al.,* 2012). It is known as *nturukpa* in Igbo language, *gbengbe* in Yoruba, *gyadar, kurmi* or *gunduru* in Hausa, *ouokisse* in French and mututi in India (Adetunji, 2007). Leaves of *P. santalinoides* are used traditionally as vegetable for making soup, and are also used in folk medicine for the treatment of various ailments, including liver diseases (Adesina, 1982).

There are a wide range of administration routes for medicines (oral, parenteral and unusual routes), which are usually chosen to optimize drug absorption (Beyddac, 1996; Kim and De Jesus, 2023). Though the oral route remains the favorite one for most drugs in many disease states, other routes are routinely used especially in veterinary practice where there are reasonable variations in the structure and function of the gastrointestinal and digestive system that may practically exclude the use of the oral route (Beyddac, 1996; AAP, 1997; Turner *et al*., 2011; Kim and De Jesus, 2023). Each route of medicine administration has its strengths and weaknesses, and the choice of any specific route should be done carefully with full consideration of the animal species to be treated, the drug, its target and the release pattern required, and also convenience and compliance (Beyddac, 1996; AAP, 1997; Turner *et al*., 2011; Kim and De Jesus, 2023).

Reports in available scientific literature have shown that oral administration of leaf extracts of *P. santalinoides* is effective in the treatment of experimentally induced liver damage in animals, and the extract has been reported to be safe for use (Anowi *et al*, 2012; Offor *et al*, 2015; Ihedioha *et al*., 2017, 2019, 2021a). There is however paucity of information in available scientific literature on the comparative hepatotherapeutic efficacy of parenterally administered methanol extracts of the leaves of *P. santalinoides* on liver damage. The aim of this study therefore was to compare the hepatotherapeutic efficacy of orally and parenterally administered methanol extracts of the leaves of *Pterocarpus santalinoides* on carbon tetrachloride (CCl_4)-induced liver damage in albino rats.

Materials and Methods

Plant collection and identification: Leaves of *P. santalinoides* used for the study were collected fresh from the plant at Nru, Nsukka Local Government Area (L.G.A) of Enugu State, Nigeria, in August 2023. The plant was identified as *Pterocarpus santalinoides* DC, Family *Papilionaceae* (*Fabaceae*) (Keay, 1989; Ogan, 2004; Adetunji, 2007), in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. A voucher specimen (UNH No. 2023/03) was deposited in the Herbarium at the University of Nigeria, Nsukka.

Experimental Animals: Thirty adult female albino rats (*Rattus norvegicus*) of twelve weeks of age were used for the study. They were sourced from the Laboratory Animal Unit of the Department of Veterinary Pharmacology and Toxicology, University of Nigeria, Nsukka, Enugu State, Nigeria. The albino rats were housed in stainless steel cages in a fly proof Animal House at room temperature (24 ºC – 28 ºC), and allowed two weeks to acclimatize before the study commenced. They were fed growers pellets (Grand Cereals Ltd, Jos, Nigeria), and provided with clean drinking water *ad libitum* all through the study. The Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine, University of Nigeria, approved the animal experiment protocol. The study followed the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC).

Drugs, Reagents and Solutions: The following drugs and reagents were used for the study: Silymarin (Sigma Aldrich, USA), Carbon tetrachloride (Sigma Aldrich, USA), Methanol (Sigma Aldrich, USA), distilled water, olive oil (Andalucia, Spain) and Clinical Biochemistry Assay Kits [Quimica Clinica Applicada (QCA) Spain].

Preparation of plant extract: Leaves of *P. santalinoides,* freshly collected from the plant, were dried under shade and ground into powder. The extract was produced by cold maceration of one kilogramme of the dried ground leaves in 80% methanol with intermittent shaking every two hours for 48 hours. The mixture was filtered with Whatman size one filter paper, the filtrate was concentrated *in vacuo* in a vacuum rotary evaporator and referred to as methanol extract of the leaves of *Pterocarpus santalinoides* (MELPS). It was stored at 4°C until time of use.

Design of the Animal Experiment: The albino rats used for the study were acclimatized for two weeks and randomly assigned into six groups (A – F) of five rats each. The rats were weighed and subacute liver damage was induced in groups A – E using carbon tetrachloride (CCl_4) at the dose of 1ml/kg in equal volume of olive oil (50 % v/v) at 3 days interval for 12 days (0, 3, 6, 9 and 12), as earlier described (Robin *et al.,* 2012; Ihedioha *et al*., 2019). Group A was given distilled water placebo at the dose of 10 ml/kg body weight (bw) and served as negative control (untreated control). Groups B, C and D were treated with methanol extract of the leaves of *Pterocarpus santalinoides* (MELPS) at the dose of 250 mg/kg bw orally, intramuscularly (IM) and intraperitoneally (IP), respectively. Group E was treated with silymarin (a known hepatoprotective drug) at the dose of 200 mg/kg bw *per os* as positive control, while Group F was treated with distilled water placebo at the dose of 10 ml/kg bw *per os* and served as normal control. Treatment was done twice daily for 5 days for all treated groups after successful induction of liver damage (after day 12 of the experiment). Blood samples (2 ml from each rat) were collected on day 0 (baseline), on day 12 (after completion of CCl_4 induction and before treatment commenced), and on day 16 (last day of the five day treatment) for serum

biochemistry (alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities, serum total protein, serum albumin, total cholesterol and total bilirubin) assay.

The serum biochemical assays followed standard biochemical procedures; it was done with a Blood Biochemistry Analyzer (Diatek Instruments Co.,Wuxi, China), using Quimica Clinica Applicada (QCA) test kits (QCA, Spain). Serum ALT and AST activities were assayed following the Reitman-Frankel method (Reitman and Frankel, 1957; Colville, 2002), while the serum ALP activity was determined by the phenolphthalein monophosphate method (Klein *et al*., 1960; Colville, 2002). Serum total protein levels was determined by the Biuret method while the serum albumin levels were determined based on the bromocresol green method (Johnson, 2008). Determination of the serum levels of total cholesterol was by the enzymatic colorimetric method (Rifai *et al*., 2008), while serum total bilirubin determination was by the Jendrassik-Grof method (Higgins *et al*., 2008). After blood sample collection on day 16 of the experiment, the rats were humanely sacrificed by intraperitoneal injection of 250 mg/kg sodium thiopentone (AVMA, 2013). The livers were eviscerated and weighed, and liver weight percentage of body weight of the individual rats was calculated.

Data analysis: Data obtained from the study were subjected to one way analysis of variance (ANOVA), to compare the different groups per unit time, and variant means were separated post hoc using the least significant difference (LSD) method. Significance was accepted at $p < 0.05$. Results were summarized as means ± standard deviation (SD) and presented in form of bar charts and tables. The data analysis was done using the SPSS software.

Results

Extraction of one kilogramme of dried ground leaves of *Pterocarpus santalinoides* yielded 103.87 grammes dried MELPS(10.39 % w/w). The MELPS was dark brown in color with a pasty consistency, and it was soluble in water.

The baseline mean values for the ALT activity of the rat groups ranged from 12.21 – 13.00 IU/L, while that of the AST activities ranged from 45.23 – 48.11 IU/L, and there were no significant variations among the groups at day 0 (baseline) (Figures 1 and 2). All the rat groups given CCl_4 (groups A, B, C, D and E) showed significantly higher ($p < 0.05$) serum ALT and AST activities on day 12 (after induction of liver damage using CCl_4), when compared to the un-induced Normal Control (Group F) [Figures 1 and 2], but on day 16 (5^{th} day of treatment with extracts) however, all the treated groups (groups B, C, D and E) had significantly lower ($p < 0.05$) serum ALT activity, when compared with the untreated negative control (Group A), with rats in group D showing the lowest serum ALT activity (Figure 1). There were no significant differences ($p > 0.05$) between the treated groups (Groups B, C, D and E) and group F (normal control) in their serum ALT activity on day 16 of the study (5^{th} day of treatment) (Figure 1).

Treatment with 250 mg/kg MELPS orally and IP, and 200 mg/kg Silymarin, respectively, led to significantly lower ($p < 0.05$) serum AST activity than those of group A (negative control) and group C (250 mg/kg MELPS IM), with group D showing the lowest level of serum AST activity on day 16 (5^{th} day of treatment) (Figure 2). There were no significant differences ($p > 0.05$) in serum AST activity between groups B, D, E and F on day 16 (Figure 2).

There were no significant variations ($p > 0.05$) in serum ALP activity of all the groups on day 0 (baseline, before CCl_4 induction) and on day 12 (after induction of liver damage using CCl_4),

but treatment with 250 mg/kg MELPS via all routes used in the study (Groups B, C and D) led to significantly higher ($p < 0.05$) serum ALP activity in the extract-treated groups when compared to group A (negative control), group E (200 mg/kg Silymarin) and group F (normal control) on day 16 of the study (5th day of treatment) [Figure 3]. There were no significant differences ($p > 0.05$) in serum ALP activity levels between groups B, C and D on day 16 (Figure 3).

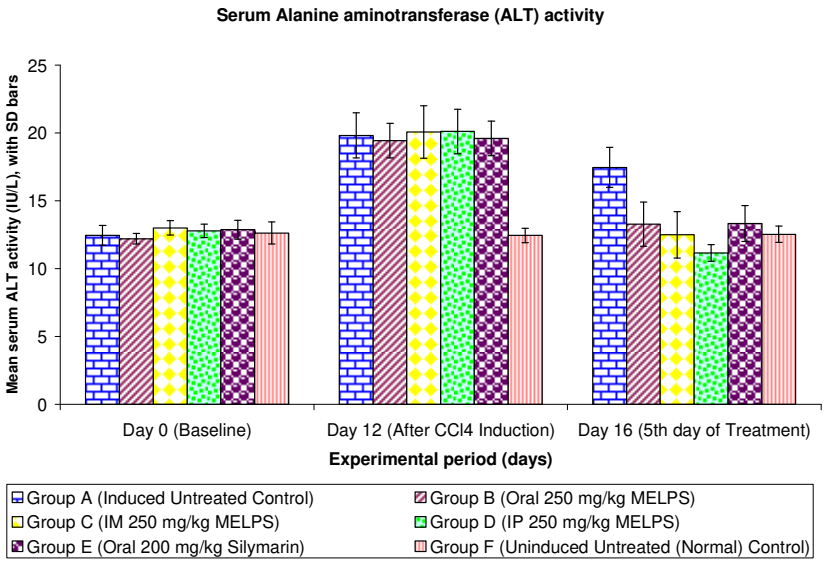

Figure 1. Serum alanine aminotransferase (ALT) activity of rat groups given CCl_4 to induce liver damage and treated with methanol extract of the leaves of *Pterocarpus santalinoides* (MELPS) by oral, intramuscular (IM) or intraperitoneal (IP) routes.

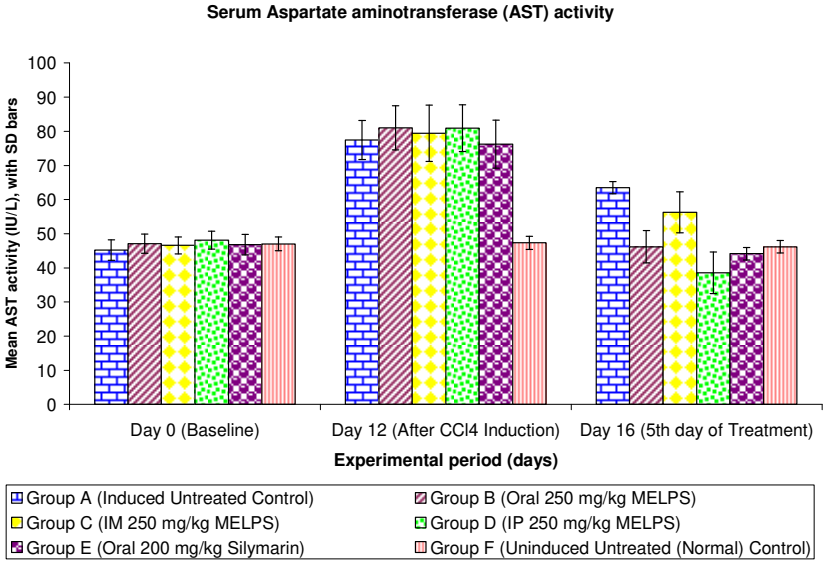

Figure 2. Serum aspartate aminotransferase (AST) activity of rat groups given CCl_4 to induce liver damage and treated with methanol extract of the leaves of *Pterocarpus santalinoides* (MELPS) by oral, intramuscular (IM) or intraperitoneal (IP) routes.

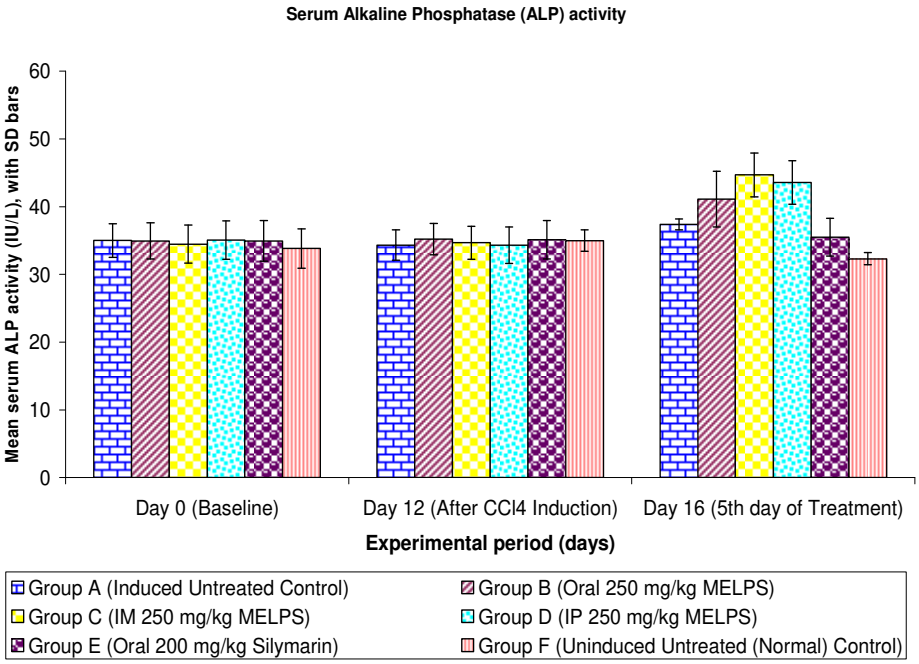

Figure 3. Serum alkaline phosphatase (ALP) activity of rat groups given CCl_4 to induce liver damage and treated with methanol extract of the leaves of *Pterocarpus santalinoides* (MELPS) by oral, intramuscular (IM) or intraperitoneal (IP) routes.

No significant ($p > 0.05$) variations were recorded among the groups in their serum levels of albumin and bilirubin all through the study (at baseline, after liver damage was induced using CCl_4 and after treatment for five days with the extracts) [Tables 1 and 2]. For the serum total protein levels, there were no significant ($p > 0.05$) variations across the groups on day 0 and day 12, but on day 16 (5^{th} day of treatment), the Group E rats that were treated with 200 mg/kg Silymarin had significantly higher ($p < 0.05$) serum total protein levels than rats in groups A, B, C and D (Figure 4). The serum total protein level of group E was comparable ($p > 0.05$) to that of group F on day 16 (Figure 4).

There were no significant variations ($p > 0.05$) in the serum total cholesterol levels across the groups on day 0 (baseline) and day 12 (after induction of liver damage with CCl_4), but treatment with 250 mg/kg MELPS via all the routes of administration used in the study led to significantly lower ($p < 0.05$) serum total cholesterol levels in the three MELPS treated groups when compared to groups A (negative control), E (200 mg/kg Silymarin) and F (normal control), with group D showing the lowest serum total cholesterol level (Figure 5).

No significant variations ($p > 0.05$) were recorded in the body weights of the rats across the groups at baseline and on day 16 (5^{th} day of treatment) [Table 3]. However, all the treated groups (Groups B, C, D and E) had relatively lower liver weight and lower relative liver weight (RLW) on day 16, though not significantly different ($p > 0.05$) from that of group A (negative control), with group D values being the lowest (Table 3). The means of the liver weight and relative liver weight of group F rats (normal control) were significantly lower ($p < 0.05$) than that of group A rats on day 16 (5^{th} day of treatment) (Table 3).

Table 1. Serum total bilirubin levels of rat groups given CCl_4 to induce liver damage and treated with methanol extract of the leaves of *Pterocarpus santalinoides* (MELPS) by oral, intramuscular (IM) or intraperitoneal (IP) routes.

Groups and their treatments	***Mean serum Total Bilirubin levels (mg/dl), with standard deviation in brackets***		
	Day 0 (Baseline)	***Day 12 (After CCl_4 Induction)***	***Day 16 (5th day of Treatment)***
Group A (Negative control)	0.47 ± 0.10	0.51 ± 0.09	0.48 ± 0.17
Group B (250 mg/kg MELPS Oral)	0.48 ± 0.08	0.46 ± 0.06	0.50 ± 0.12
Group C (250 mg/kg MELPS Intramuscular)	0.50 ± 0.10	0.48 ± 0.08	0.52 ± 0.07
Group D (250 mg/kg MELPS Intraperitoneal)	0.47 ± 0.07	0.46 ± 0.10	0.55 ± 0.13
Group E (200 mg/kg Silymarin)	0.46 ± 0.08	0.46 ± 0.10	0.40 ± 0.10
Group F (Normal Control)	0.47 ± 0.10	0.48 ± 0.08	0.43 ± 0.10

No significant differences between the groups ($p > 0.05$) all though the study

Table 2. Serum albumin levels of rat groups given CCl_4 to induce liver damage and treated with methanol extract of the leaves of *Pterocarpus santalinoides* (MELPS) by oral, intramuscular (IM) or intraperitoneal (IP) routes.

Groups and their treatments	***Mean serum Albumin levels (g/L), with standard deviation in brackets***		
	Day 0 (Baseline)	***Day 12 (after CCl_4 induction)***	***Day 16 (5^{th} day of treatment)***
Group A (Negative control)	38.26 ± 3.61	39.62 ± 4.16	38.88 ± 2.64
Group B (250 mg/kg MELPS Oral)	37.93 ± 3.00	38.24 ± 3.83	39.89 ± 1.97
Group C (250 mg/kg MELPS Intramuscular)	39.41 ± 2.67	38.21 ± 3.86	39.45 ± 1.28
Group D (250 mg/kg MELPS Intraperitoneal)	37.42 ± 3.66	37.93 ± 4.01	38.61 ± 2.41
Group E (200 mg/kg Silymarin)	38.36 ± 4.01	39.02 ± 3.26	42.77 ± 2.15
Group F (Normal Control)	39.81 ± 4.26	40.09 ± 3.16	38.19 ± 3.18

No significant differences between the groups ($p > 0.05$) all though the study

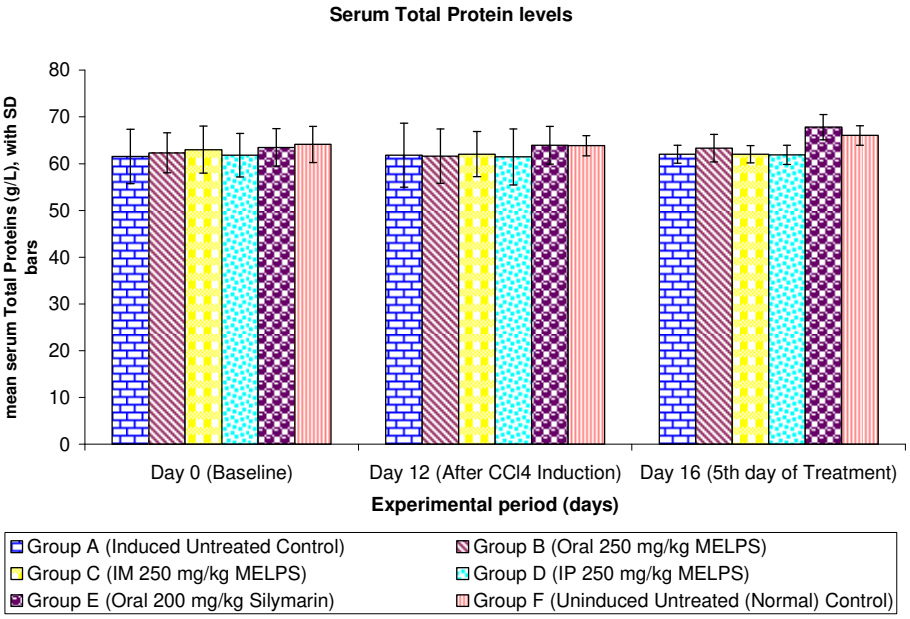

Figure 4. Serum total protein levels of rat groups given CCl_4 to induce liver damage and treated with methanol extract of the leaves of *Pterocarpus santalinoides* (MELPS) by oral, intramuscular (IM) or intraperitoneal (IP) routes.

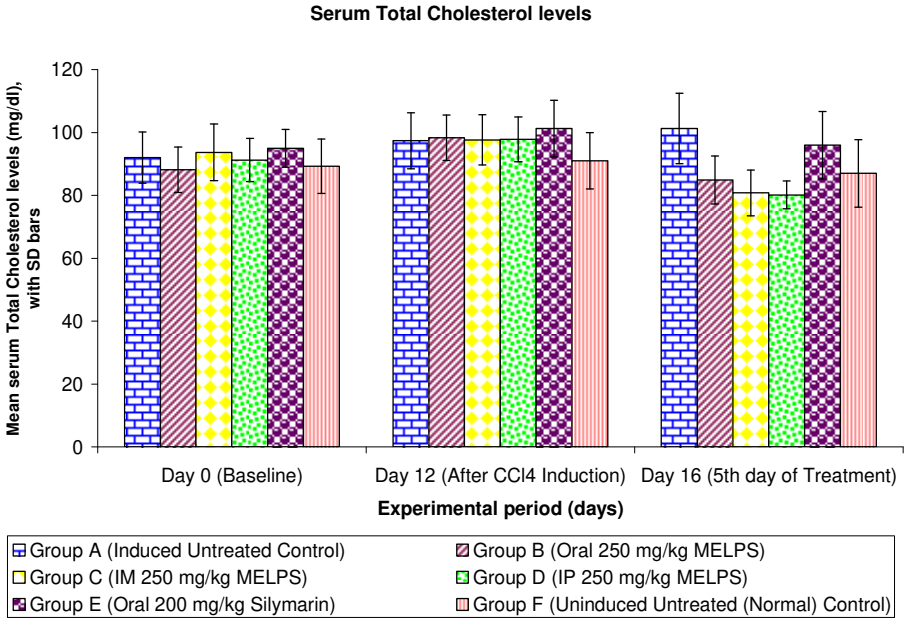

Figure 5. Serum total cholesterol levels of rat groups given CCl_4 to induce liver damage and treated with methanol extract of the leaves of *Pterocarpus santalinoides* (MELPS) by oral, intramuscular (IM) or intraperitoneal (IP) routes.

Table 3. Body weight, liver weight and relative liver weight of rat groups given CCl_4 to induce liver damage and treated with methanol extract of the leaves of *Pterocarpus santalinoides* (MELPS) by oral, intramuscular (IM) or intraperitoneal (IP) routes.

Groups and their treatments	*Means, with standard deviation in brackets*		
	Day 16 Body weight (g)	*Liver weight (g) on day 16*	*Relative Liver weight (%) on day 16*
Group A (Negative control)	188.67 ± 4.93	9.62 ± 0.85 [a]	5.11 ± 0.59 [a]
Group B (250 mg/kg MELPS Oral)	183.00 ± 15.13	8.81 ± 2.07 [ab]	4.62 ± 0.57 [ab]
Group C (250 mg/kg MELPS Intramuscular)	192.33 ± 6.03	9.56 ± 1.73 [ab]	4.97 ± 0.72 [ab]
Group D (250 mg/kg MELPS Intraperitoneal)	173.33 ± 29.69	8.49 ± 1.91 [ab]	4.90 ± 0.25 [ab]
Group E (200 mg/kg Silymarin)	186.00 ± 14.42	8.51 ± 1.24 [ab]	4.57 ± 0.48 [ab]
Group F (Normal Control)	180.25 ± 24.76	7.38 ± 0.95 [b]	4.11 ± 0.47 [b]

[a, b] Different alphabetical superscripts in a column indicate significant difference ($p < 0.05$) between the mean values of the groups on the specified experimental day.

Discussion and Conclusion

The significantly higher serum ALT and AST activities in Groups A, B, C, D and E on day 12 is a confirmation of the ability of CCl_4 to damage hepatocytes and disrupt/alter hepatocellular integrity leading to leakage of hepatocyte bound enzymes into the general circulation (Singh *et al*., 2011). The findings in this study on day 16 that rats in groups B, C and D had their serum ALT and AST activities lower than that of Group A (negative control), and comparable to that recorded for the silymarin treated positive control (Group E) and the normal control (Group F), implied that administration of 250 mg/kg MELPS given by oral, IM and IP routes was able to restore the hepatocellular integrity of the damaged liver and compared effectively with 200 mg/kg silymarin (a known oral hepatoprotective drug). This suggests that intramuscular and intraperitoneal routes of administration of MELPS are as efficacious as the oral route, with intraperitoneal route being the most efficacious. Such reductions in serum ALT and AST activities were earlier reported when MELPS was administered orally to CCl_4-induced liver damage in albino rats (Ihedioha *et al*., 2019, 2021b, 2022, 2023). The serum AST activity recorded in group C rats treated with 250 mg/kg MELPS via the intramuscular route was not significantly different from that of group A rats (negative control), probably because AST is a marker of both muscle and liver damage, and the intramuscular injections may have led to some form of muscle injury.

The administration of carbon tetrachloride to induce liver damage had no observable effect on ALP activity in all the groups given CCl_4 on day 12, but treatment with MELPS via all the routes of administration (Groups B, C and D) led to significantly higher serum ALP activity than what was recorded for groups A, E and F

on day 16, suggesting that MELPS may be an ALP inducer. Some drugs are known to be associated with induction of the secretion of alkaline phosphatase (Balazs *et al*. 1978; Fux *et al*., 2008; Siddique and Kowdley, 2012; Nabil *et al*., 2022; Francis and Navarro, 2024): this is not considered a toxic effect but may be part of the beneficial pharmacological action of these drugs. The findings in the present study of significantly higher serum ALP activity in the groups treated with MELPS concurs with the reports of previous studies on the plant extract (Offor *et al*., 2015; Ihedioha *et al*., 2017, 2019, 2021b).

Carbon tetrachloride administration and treatment with MELPS via all the routes used in the study had no significant effect on serum levels of bilirubin, total proteins and albumins, suggesting that sub-acute administration of CCl_4 and treatment with MELPS as used in this study did not affect the hepatobiliary and protein synthetic functions of the liver (Thapa and Walia, 2007; Yap and Aw, 2010; Ihedioha *et al*., 2021a). The significantly higher serum total protein levels recorded for the group treated with Silymarin in the present study concurs with earlier reports of the ability of Silymarin administration to enhance overall protein synthesis (Pradhan SC and Girish C, 2006; Karimi *et al*., 2011; Vargas-Mendoza *et al*., 2014).

The significantly lower serum cholesterol levels recorded on day 16 (5^{th} day of treatment with extracts) for the rat groups treated with MELPS via all the routes of administration (Groups B, C and D) is in agreement with earlier published reports on the ability of leaf extracts of *Pterocarpus santalinoides* to lower serum cholesterol levels (Offor *et al*., 2015; Ihedioha *et al*., 2017, 2019, 2021b, 2022). It also shows that the parenteral routes of administration compared effectively with the oral route of administration in lowering the serum cholesterol levels.

The higher liver weights and relative liver weights in groups A, B, C, D, and E following CCl_4 administration is an indication of hepatomegaly and degeneration caused by CCl_4 hepatotoxicity (Tahashi *et al.,* 2002; Bukhsh *et al*., 2014). The lowering of the liver weight and relative liver weight recorded in Groups B, C, D and E after treatment with 250 mg/kg MELPS via all the routes of administration and 200 mg/kg silymarin respectively, though not significantly different from that of the negative control (Group A), implied amelioration of the hepatomegaly induced by CCl_4 toxicity, and also showed that parenteral routes compared effectively with the oral route of administration in ameliorating the hepatomegaly. This lowering of liver weight and relative liver weight in the MELPS and silymarin treated groups as recorded in the present study concurs with earlier reports by Ihedioha *et al*. (2017, 2019, 2021b), and may be attributed to the presence of the phytochemical constituents of this plant extract such as flavonoids and tannins which are natural antioxidants (Bothon *et al*., 2014; Ihedioha *et al*., 2017, 2018, 2019; Enemali *et al.,* 2019). Reports by other researchers have shown that antioxidants mop-up reactive oxygen species (ROS) and ameliorate damage to the liver (Karahan *et al.,* 2005; Valko *et al;* 2007).

Based on the results of the study, it was concluded that intramuscular and intraperitoneal routes of administration as used in this study compared effectively with the oral route, and that the intraperitoneal route, especially, even led to better (more efficacious) hepatotherapeutic effect than the oral route. Thus, *Pterocarpus santalinoides* methanol leaf extract can be effectively administered parenterally as a hepatotherapeutic agent.

Acknowledgement

The authors acknowledge the laboratory support of the Biomedical Research Support Unit of the Foundation for Education and Research on Health, Nsukka Nigeria, for the clinical biochemistry assay.

Conflict of interest

The authors declare no conflict of interest associated with this work.

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