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Original Article

Effect of n-butanol fraction of *Detarium microcarpum* stem bark on some liver function and oxidative stress parameters in carbon tetrachloride induced hepatic injury in Wistar rats

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Abstract

Hepatic damage is a serious health challenge that alters normal metabolic processes in the body. Total flavonoid, phenolic content and lethal dose (LD_{50}) of n-butanol fraction of *Detarium microcarpum* stem bark were evaluated using standard procedures. The effect of n-butanol fraction of *Detarium microcarpum* stem bark on the relative liver weight, some liver functions (Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin (Bil)) and oxidative stress (malondialdehyde MDA, superoxide dismutase SOD, and catalase CAT) parameters in carbon-tetrachloride (CCl_4) induced hepatic injury in Wistar rats were investigated. Experimental animals were divided into 6 groups, to induce hepatic injury. CCl_4 in olive oil (1:1) was introduced by intraperitoneal injection once a week for 4 weeks and graded doses (100, 150 and 200) mg/ml of n-butanol fraction of *Detarium microcarpum* stem bark were orally administered, daily for 28 days. The quantity of total flavonoids and phenols in n-butanol fraction of *Detarium microcarpum* stem bark were 234.42 ± 0.71 and 2.97 ± 0.31 (mg/g) respectively. The LD_{50} of n-butanol fraction of *Detarium microcarpum* stem bark was >5000 mg/kg body weight of rats. The relative liver weight of all the treated groups decreased significantly (p<0.05) compared to the CCl_4 only treatment (negative control). ALT, AST, ALP, Bil and MDA significantly decreased while SOD and CAT increased significantly compared with the negative control. These results suggest that *Detarium microcarpum* stem bark contains phytochemicals with antioxidant properties and holds promise in ameliorating liver injuries.

Keywords: *Detarium microcarpum*, hepatotoxicity, carbon-tetrachloride, liver function, oxidative stress

1. Introduction

The liver is the second largest organ in the body (Pandian, Badami, & Shankar, 2013). It controls several important functions in the body, such as detoxification, metabolism of drugs, synthesis of plasma proteins and excretion. Any form of toxic injury to the liver could lead to a series of metabolic reactions affecting its normal functions/functioning. With reference to the total world population, hepatic disease has become common not only in the Western world but also in developing countries (Vidona & Wadioni, 2018). Carbon tetrachloride (CCl_4) is an effective and well-known toxic chemical used in experimental animals to induce liver injuries. This chemical produces free radicals in body tissues and fluids, which source for electrons to stabilise themselves, and in so doing, disrupt proteins, lipids and some enzymatic activities in the body. *Detarium microcarpum* is an African tree commonly known as "sweet dattock" or "tallow tree", belonging to the

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family Fabaceae (legumes). It is most common in wooded savannahs, semi-cleared dry forest areas and fallows, growing in sandy or hard soils with high iron content (Kouyaté & Lamien, 2011). In Nigeria, it is known as Taura (Hausa), and as Gungorochi, (Nupe). The plant is used to treat diseases such as tuberculosis and meningitis (Abdulbasit, Mohamed, Mir-ghani, Bustamam, & Siddig, 2011). Pharmacological studies on the bark of *Detarium microcarpum* include reports of antiviral (Olugbuyiro, Moody, & Hamana, 2009), antibacterial (Garba, Andrew, & Gayus, 2013) and anti-diarrhoeal (Tijani, Barkindo, Ngulde, Wampana, & Sanda, 2013) activities. The bark of *Detarium microcarpum* is used traditionally in Nigeria as a valuable remedy to treat liver disease, hence the present study was undertaken to investigate its effects on some liver functions and oxidative stress parameters after hepatic damage was induced in rats by the industrial toxicant carbon tetrachloride (CCl_4).

2. Materials and Methods

2.1 Plant materials and extractions

The stem bark of *Detarium microcarpum* was collected in the month of April from Minna, Niger State, and identified by a taxonomist at the Herbarium unit in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

2.2 Extraction

Air dried stem bark powder (approximately 1 kg) was soaked in 2.5 L distilled water for 48 h, with occasional shaking. The suspension was filtered using muslin cloth and passed through Whatman filter paper No 1. The filtrate was evaporated to dryness in a water bath at 45 °C and stored in airtight containers till further use.

The crude extract of *D. microcarpum* stem bark was reconstituted with water (200 ml) and subjected to liquid-liquid partitioning with ethyl acetate and n-butanol solvents se-quentially as a 1:1 (v/v) solution (Abbot & Andrew, 1970). The process was repeated thrice to obtain the respective fractions.

2.2.1 Total flavonoid content

Total flavonoid content of n-butanol fraction of *D. microcarpum* stem bark was determined by aluminium chloride colorimetric assay (Olajire & Azeez, 2011).

2.2.2 Total phenolic assay

Total phenolic content of n-butanol fraction of *D. microcarpum* stem bark was determined by Folin-Ciocalteu method (Chun, Kim, & Lee, 2003).

2.3 Acute toxicity test

Acute toxicity test for n-butanol fraction followed the method described by Lorke (1983) to select a suitable dose for the study. The rats were divided into 6 groups of 3 rats each and administered by gavage the doses (10, 100, 1000, 1600, 2900 or 5000) mg/kg body weight. They were observed for 24 hours for signs of toxicity, including death.

2.4 Induction of liver damage

The induction of liver damage was performed by intraperitoneal injection of CCl_4 in olive oil (1:1) once weekly for a period of four weeks. After each induction, animals were fasted for 36 hours before commencement of treatment.

2.5 Experimental animals

Wistar albino rats weighing 100 - 150 g were ob-tained from the animal house, Department of Pharmacology, Ahmadu Bello University, Zaria, Kaduna State, for the study. The animals were allowed to acclimatize for two weeks at 26±2 ^{o}C room temperature and were kept under twelve hours of dark/light cycle with free access to feed and water ad-libitum.

Thirty-six rats divided into six groups of six rats each were designated as groups A-F. Groups B-F were treated to induce hepatic damage. Group A served as the normal control, Group B was administered CCl_4 only, and groups C-E were treated orally with graded (100, 150 and 200) mg/kg body weight doses of n-butanol fraction of *Detarium microcarpum* stem bark. Group F was treated with a standard drug (silymarin, 100 mg/kg). Treatment was done once daily for 28 days, after which the rats were sacrificed by cardiac puncture using chloroform as anaesthesia and blood samples were collected for biochemical analysis. The liver was harvested and weighed, and sampled for determining oxidative stress parameters. The relative liver weight of each rat was calculated as follows:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

2.6 Liver function parameters

At the end of the study on the 28th day, the blood samples collected into non heparinised tubes were centrifuged at 3000 rpm for 10 minutes. The serum was separated and analysed. Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) were assayed using the Reithman and Frankel (1957) method; serum alkaline phosphatase (ALP) was analysed by the method of Haussament (1977); serum bilirubin concentration by the method of Jendrassik and Grof (1938), total protein by the method of Lowry, Rosebrough, Farr, and Randall (1951) and serum albumin concentration by Doumas, Watson, and Homer (1997) methods, respectively.

2.7 Preparation of liver homogenate

One gram (1 g) of the liver was crushed and homogenized in 10 ml of 50 mM sodium phosphate buffer (pH 7.4). This was centrifuged at 4000 rpm for 10 minutes, and then the supernatant was collected using a Pasteur pipette for oxidative stress parameter determinations.

2.8 Oxidative stress parameters

The determinations were done for catalase (CAT) activity (Aebi, 1984), superoxide dismutase (SOD) activity (Martin, Dailey, & Sugarman, 1987) and thiobarbituric acid re-active substance (TBARS) (Fraga, Leibovitz, & Tappel, 1988).

2.9 Statistical analysis

The data were subjected to analysis of variance (ANOVA) using SPSS 20.0 for Windows software. The results are expressed as mean ± standard deviation (SD). Significant differences between the treatment groups were called by using the Duncan Multiple Range Test (Duncan, 1955). A probability level of less than 5 % (p<0.05) was considered significant.

3. Results

3.1 Total flavonoids and total phenol content

The quantity of total flavonoids in n-butanol fraction of *D. microcarpum* stem bark was 234.42 ± 0.71 mg/g while for total phenol was 2.97 ± 0.31 mg/g (Table 1).

Table 1. Total flavonoid and Phenolic content in n-butanol fraction of *Detarium microcarpum* stem bark.

Fraction	TFC(mg/g)*	TPC(mg/g)**
BF	234.42±0.71	2.97±0.31

Values expressed as mean ± standard deviation of three (3) determinations. * mg quercetin/g fraction, ** mg gallic acid/g fraction. TFC: total flavonoid content, TPC: total phenolic content, BF: n-butanol fraction

3.2 Acute toxicity test

The acute toxicity studies revealed that the n-butanol fraction of *D. microcarpum* stem bark was safe up to 5000 mg/kg body weight. However, at higher doses behavioural changes were observed in the rats, such as isolation, increased breathing rate, and scratching, which lasted for about 3 hours, after which the animals resumed and maintained their normal activities. Therefore, the LD_{50} value was estimated to be greater than 5000 mg/kg.

3.3 Effects on liver weight

The relative weight of the liver increased significantly (p<0.05) in Group B (CCl_4 only). Treatment with n-butanol fraction significantly (p<0.05) decreased the weight of the liver in all the treated groups, compared to Group B (CCl_4 only), Table 2.

Table 2. Effect of n-butanol fraction of *Detarium microcarpum* stem bark on relative liver weight of CCl_4 induced hepatic damage in Wistar rats.

Group	Treatment	Liver (%)
A	Normal control	3.4 ± 0.35^{a}
B	CCl_4 only	4.8 ± 0.29^{d}
C	CCl_4 + 100 mg/kg *D.microcarpum*	3.8 ± 0.22^{abc}
D	CCl_4 + 150 mg/kg *D.microcarpum*	3.9 ± 0.46^{bc}
E	CCl_4 + 200 mg/kg D.microcarpum	3.9 ± 0.25^{bc}
F	CCl_4 + 100 mg/kg silymarin	3.6 ± 0.36^{abc}

Values expressed as mean ± Standard deviation, Values with different superscripts down the column differ significantly (p<0.05).

3.4 Liver function parameters

The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) with direct bilirubin (DB) and indirect bilirubin (IB) concentrations in the treated groups were significantly (p<0.05) reduced in comparison to Group B (CCl_4 only) (Figures 1 and 2). The result for Group D (150 mg/kg bodyweight of n-butanol fraction) is comparable to that of the silymarin treated group (Group F). The n-butanol fraction of *Detarium microcarpum* stem bark had no significant effect on serum total protein (TP) or albumin (ALB), Figure 3.

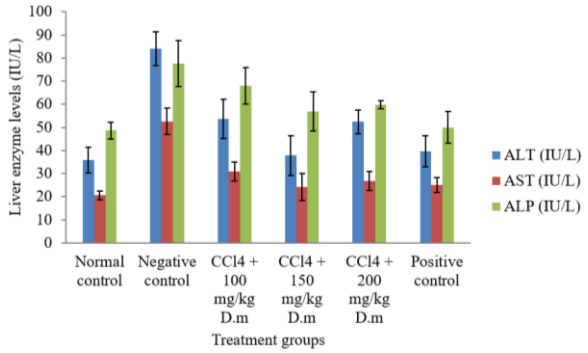

Figure 1. Effects of n-butanol fraction of *D. microcarpum* stem bark on serum liver enzymes of CCl_4 induced hepatic damage in Wistar rats.

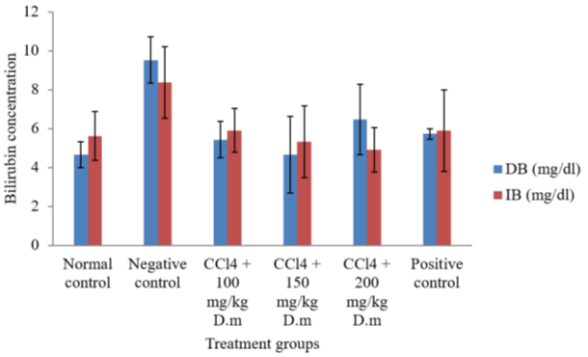

Figure 2. Effect of n-butanol fraction of *D. microcarpum* stem bark on direct bilirubin (DB) and indirect bilirubin (IB) of CCl_4 induced hepatic damage in Wistar rats.

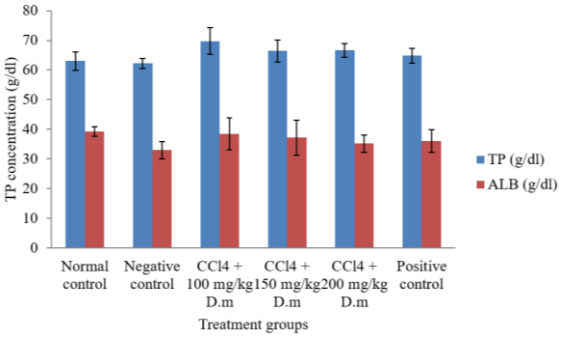

Figure 3. Effect of n-butanol fraction of *D. microcarpum* (*D.m*) stem bark on total protein (TP) and albumin (ALB) of CCl_4 induced hepatic damage in Wistar rats.

3.5 Oxidative stress parameters

The activities of superoxide dismutase (SOD), and catalase (CAT) were significantly increased while malondialdehyde (MDA) was significantly decreased in all the treated groups compared with Group B (CCl_4 only). Group D treated with 150 mg/kg body weight showed a comparable result to that of the silymarin treated rats (Group F), Figures 4, 5 and 6.

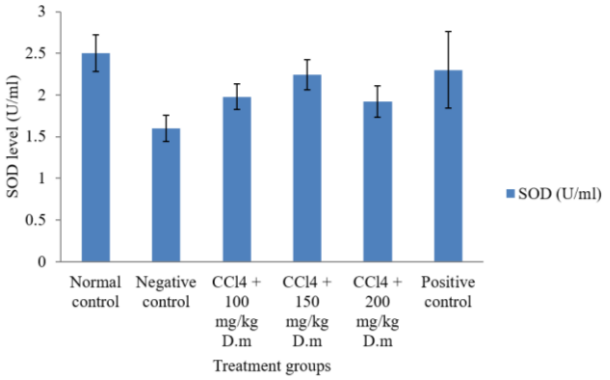

Figure 4. Effect of n-butanol fraction of *D. microcarpum* (*D.m*) stem bark on superoxide dismutase (SOD) of CCl_4 induced hepatic damage in Wistar rats.

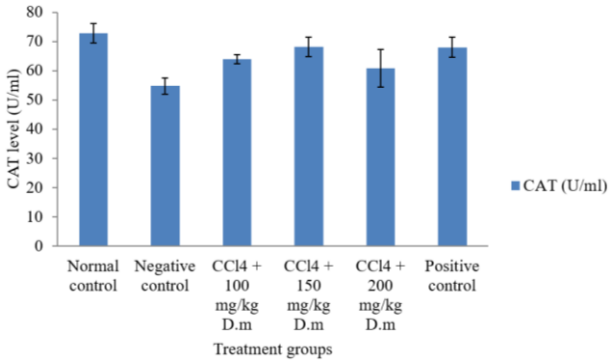

Figure 5. Effect of n-butanol fraction of *D. microcarpum* (*D.m*) stem bark on catalase (CAT) of CCl_4 induced hepatic damage in Wistar rats.

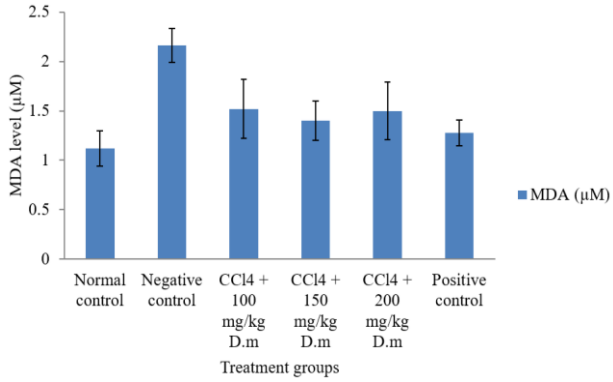

Figure 6. Effect of n-butanol fraction of *D. microcarpum* (*D.m*) stem bark malondialdehyde (MDA) of CCl_4 induced hepatic damage in Wistar rats.

4. Discussion

The results from quantitative phytochemical analysis of n-butanol fraction of *D. microcarpum* stem bark showed the total flavonoids content to be higher than the total phenolic content. Flavonoids and phenolic compounds are plant derived phytochemicals important for growth, and serve as natural antioxidants that can be beneficial to human health in curing and preventing many diseases (Tungmunnithum, Thongboonyou, Pholboon, & Yangsabai, 2018).

The oral minimum lethal dose (LD_{50}) of n-butanol fraction of *Detarium microcarpum* stem bark was found to be above 5000 mg/kg body weight of rats, indicating low toxicity. Earlier reports stated that LD_{50} of substances administered by oral intubation estimated above 2000 mg/kg body weight could be considered of low toxicity and safe, as cited in Ogbe, Adenkola, and Anefu (2012). The behavioural changes such as increased breathing rate and scratching observed at higher doses of the fraction may be due to one or more irritating compounds in the n-butanol fraction of *Detarium microcarpum* stem bark (Taziebou, Etoa, Nkegoum, Pieme, & Dzeufiet, 2007).

The increase in liver weight observed in Group B (CCl_4 only) may be due to the toxic effects of CCl_4 in the liver. Increase or decrease in either absolute or relative weight of an organ after administration of a chemical or drug is an indication of the toxic effects of that chemical (Orisakwe, Udemezue, & Orish, 2003). The administration of n-butanol fraction significantly reduced the liver weight of rats in all the treatment groups, maybe as a result of the high flavonoid content of the fraction. According to Li *et al.* (2018), flavonoids possess anti-inflammatory properties.

The hepatic damage increased the levels of ALT, AST and ALP in the serum suggesting that the hepatic injury caused by CCl_4 released these liver enzymes into the blood. However, treatment with n-butanol fraction significantly reduced the blood levels of these liver enzymes, possibly by the antioxidant potential of flavonoids contained in the fraction to inhibit enzymes involved in free radical generation, and thus, improved cell viability and stopped cellular leakage of ALT, AST and ALP in the hepatocytes (Wu *et al.*, 2006). According to Dakrory, Fahmy, Soliman, Mohamed, and Amer (2015), antioxidants are responsible for the repair and maintenance of cellular membrane integrity. The liver is responsible for the removal of bilirubin from the body, so any form of injury to it could distort its normal functioning, leading to accumulation in the blood. Thus, a high level of bilirubin is used as an index for liver function and bile excretion status (Usha, Mary, & Hemalatha, 2008). The high levels of bilirubin were however reduced significantly ($p<0.05$) after treatment with n-butanol fraction, suggesting that the antioxidant potential of n-butanol fraction of *D. microcarpum* stem bark enhanced hepatic functions. This result is in line with the prior report by Ansah, Dadzeasah, and Asiamah, (2013).

Synthesis of serum albumin and total protein are affected during hepatic damage, leading to their reduction. In this study, a non-significant decrease in total protein and albumin observed in the Group B (CCl_4 only) may have been due to inflammation of the liver. This inflammation may have affected synthesis of total protein and serum albumin. Earlier studies reported a non-significant ($p>0.05$) reduction effect on protein level in CCl_4 induced hepatotoxicity, which suggested

that probably the liver damage was acute and the reserve capacity of the liver coupled with the relative long half-life of these proteins did compensate for the damage (Etuk, Agaie, Ladan, & Garba, 2009).

Lipid peroxidation is associated with oxidative stress initiated by CCl_4 administration (Augustyniak, Wazkilwicz, & Skrzydlewaka, 2005). In the present study, CCl_4 induced rats showed a significant increase ($p<0.05$) in MDA level, which was also reported by Abdou, Saleh, & Khalil, (2015). MDA activity in the treated groups was significantly reduced, and flavonoids are responsible for decreased lipid peroxidation and oxidative stress by mechanisms such as iron-chelating and iron-stabilizing (Vieira *et al.*, 2011).

Decreased levels of serum SOD and CAT activities observed in the negative control group may be due to the increased actions of reactive oxygen species in the liver (Gamal, Kadry, Mohamed, & El-Shymaa, 2016). However, these enzymatic activities were increased in the n-butanol fraction treated groups, which could be a result of the anti-oxidant properties of flavonoids in the fraction, known to modulate the activities of different enzymes due by interactions with various biomolecules (Kumar, Sharma, Sourirajan, Khosla, & Dev, 2018). From the results above, it was observed that 150 mg/kg body weight of n-butanol fraction of *Detarium microcarpum* stem bark was the most effective dose in treating hepatic injury, compared to 100 of 200 mg/kg body weight dose levels of n-butanol fraction. This may be caused by the presence of a compound in n-butanol fraction of *Detarium microcarpum* stem bark, which may be responsible for the substitution or blockage of hydroxyl groups at carbon position 3 of flavonoids at higher doses of the fraction. The substitution may have rendered flavonoids less reactive and decreased the antioxidant properties, since increased degree of hydroxylation of flavonoids is reported to increase their antioxidant activity (Russo, 2018).

5. Conclusions

The n-butanol fraction of *Detarium microcarpum* stem bark was found to be a rich source of flavonoids. The therapeutic effect of the fraction may involve its antioxidant abilities in reducing the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) enzymes, and in maintaining the integrity of the cell membrane. The increases in catalase and superoxide dismutase enzymes prevented excessive accumulation of free radicals in the liver. This antioxidant effect of *Detarium microcarpum* stem bark could be of interest in drug development.

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