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Biochemical Effect of Aqueous Leaf Extract of *Detarium microcarpum* in Wistar Rats

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Abstract

Herbal remedies have been used since ancient times, often sourced from local traditional healers. However, many of these preparations are administered without thorough scientific evaluation. This study aimed to assess the effects of aqueous leaf extract of *Detarium microcarpum* on liver and kidney function in Wistar rats. A total of 25 male rats were randomly assigned to five groups of five animals each. Groups 2, 3, 4, and 5 received oral doses of 200, 400, 600, and 800 mg/kg body weight of the extract, respectively, for 21 consecutive days. Group 1, serving as the control, received only normal saline. Liver function was evaluated by measuring serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, and albumin. All liver parameters exhibited a significant dose-dependent increase ($p<0.05$) in the treatment groups compared to the control, indicating hepatotoxic effects. Similarly, kidney function parameters, including bicarbonate (HCO_3^-), chloride (Cl^-), sodium (Na^+), and potassium (K^+), also showed a significant dose-dependent increase. Conversely, levels of urea and creatinine significantly decreased ($p<0.05$) in the extract-treated groups relative

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to the control. The findings suggest that the aqueous leaf extract of *D. microcarpum* adversely affects liver and kidney function in a dose-dependent manner. Therefore, despite its traditional use, the extract should be considered potentially toxic and used with caution.

Keywords: Kidney; Liver; *Detarium microcarpum*; Total Protein; Albumin

INTRODUCTION

Medicinal plants serve as a reservoir of plant-based drugs and natural products with diverse pharmacological activities (Musa *et al.*, 2024; Abaka *et al.*, 2024). Moreover, their abundance, affordability, and accessibility provide an alternative for managing various ailments (Dahiru *et al.*, 2024). Herbal remedies represent the main treatments used over millennia for preventing and treating a wide variety of ailments (Petran *et al.*, 2024). Since ancient times, people have prepared their herbal medicines or acquired them from local traditional healers (Che *et al.*, 2024). It is highly suggestive that about 80% of the population in developing countries still use herbal medicine to meet their primary healthcare needs (Zhang *et al.*, 2019). Furthermore, in the past few decades, people have been rediscovering more traditional and predominantly herbal medicine (Murgia *et al.*, 2021), either as an alternative to or in conjunction with modern drugs (Santucci *et al.*, 2021). Many herbal remedies obtained from plants are processed and administered without scientific evaluation of their safety (Ahmed *et al.*, 2022).

D. microcarpum, a perennial tree, also called small detar or sweet detar, can grow up to 10 m tall and occurs naturally in the arid regions of West and Central Africa (Kouyate and Van Damme, 2006; Rouamba et al., 2016). It is found in shrub savannas, wooded savannas, open forests, and dry forests, as well as in fallows (Arbonnier, 2009). It generally grows on marginal soils such as sandy and lateritic soils (Kouyate, 2005), thereby involving little to no land use competition with crops. It reproduces through rejection, suckers, and spontaneously sprouted seeds (Kouyate, 2005). This capacity suggests the possibility of vegetative propagation of *D. microcarpum*, which is necessary for its domestication. Regarding the species' growth, it can reach a height of 50 cm after one year and 120 cm after seven years (Bastide and Ouedraogo, 2008). It is also a species that withstands stress, such as cutting (Bationo *et al.*, 2001; Sawadogo *et al.*, 2002). In folklore medicine, *D. microcarpum* is considered a potent medicinal herb and is traditionally used to cure and

prevent many diseases, including oxidative stress-related ailments such as cancer. Scientific studies have shown that *D. microcarpum* possesses antimicrobial, hepatoprotective, cytotoxic, and antidiabetic effects (Shofian *et al.*, 2011; Hamza *et al.*, 2014; Rouamba *et al.*, 2016). This study, however, aimed to evaluate the effects of *D. microcarpum* aqueous leaf extract on liver and kidney function parameters.

MATERIALS AND METHODS

Plant material

D. microcarpum was collected from a Vimtim community in the Mubi North Local Government Area, Adamawa State, Nigeria. The plant material was taxonomically identified by a botanist from the Department of Botany, Adamawa State University, Mubi, where the herbarium voucher was deposited.

Laboratory animals

Twenty-five male Wistar rats weighing between 120 and 150 g used for the experiment were obtained from the Animal House of Adamawa State University, Mubi. The animals were acclimatized for one week, and all animals were treated in a manner that complied with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (NIH publication, 1985). The animals were allowed free access to grower mash and water where a 23 -25^{0}C temperature and a 12 h light/dark light cycle was maintained throughout the experiment period. An ethical clearance for experimenting on research animals was secured from the University Ethical Committee before the initiation of the experiment with an approval number of …….

Plant extraction

The leaves of *D. microcarpum* were washed and air dried for 14 days, after which it was pulverized into fine powder. The method used for the extraction was as described by Adebayo *et al.* (2006). The powdered leaves of *D. microcarpum* (800 g) were soaked in 8 L of distilled water for four days, after which the extract was filtered using a Whatman no. 1 filter paper. It was further concentrated at 50°C using a rotary evaporator and further concentrated using a water bath at 48°C. The weight of the extract obtained was 67 g, giving a percentage yield of 8.9%.

Experimental design

Twenty-five (25) male albino Wistar rats were used for the study. The rats were divided into four groups of seven rats per group. The crude extract was dissolved in normal saline (0.9%) before the treatment. Groups 1, 2, 3, 4, and 5 were used for the sub-chronic experiment. Groups 2, 3, 4, and 5 were, respectively, administered with 200, 400, 600, and 800 mg/kg body weight of aqueous leaf extract of *D. microcarpum* for 21 days orally. Animals in group 1 (control group) did not receive the extract, but were treated with normal saline. After this period, the rats were subjected to an overnight fast and were subsequently anaesthetized with diethyl-ether, and the blood sample was collected by cardiac puncture into EDTA and were analyzed for liver and kidney parameters based on the method described by Rietman and Frankel (1957), Wright *et al.* (1972), Penhaker *et al.* (2013), and Rifai (2018).

RESULTS

The phytochemical composition of aqueous *D. microcarpum* leaf extract are presented in Table 1. The phytochemicals assayed for include alkaloids, flavonoids, tannins, saponins, and sterols. The result disclosed the presence of all the phytochemicals assayed for

Table 1. Qualitative phytochemical constituents of the aqueous leaf extract of *D. micropapum*

Phytochemical	Inference
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Sterols	+

Key: + = present

Table 2 showed the serum liver indices of rats treated with the aqueous D. microcarpum leaf extract. The total protein levels increased significantly ($P<0.05$) across all the treatment groups in a dose dependent manner and when compared to the control group. Albumin levels showed a significant increase ($p<0.05$) in a dose dependent manner and when compared to the control groups, however the groups treated with 200 and 300 mg/kg body weight of the extract were not significantly different ($p<0.05$). There was a dose dependent

significant increase (p<.0.5) in ALT levels when the treatment groups were compared and when compared to the control groups but groups that received 200 and 400 mg/kg body weight of the extract were not significantly different (p<0.05). AST levels showed significant decrease (p<0.05) in all the groups in a dose dependent manner and when compared to the control group. ALP levels showed significant increase (p<0.05) dose dependently and when compared to the control group, howbeit, groups that were treated with 600 and 800 mg/kg body weight of the extract were not significantly different (p<0.05).

Table 2. Serum liver indices of rats administered with aqueous leaf extract of *D. microcarpum*

Group	T. protein (g/dL)	Albumin (g/dL)	ALT (UL)	AST (UL)	ALP (UL)
Control	63.00 ± 0.61^{a}	38.33 ± 0,85^{a}	33.67 ± 1.53^{a}	53.00 ± 1.00^{a}	64.67 ± 1.53^{a}
200 mg/kg b.wt.	82.33 ± 0.91^{b}	56.87 ± 0.15^{b}	52.33 ± 1.53^{b}	154.67 ± 0.58^{b}	151.32 ± 1.15^{b}
400 mg/kg b.wt.	84.97 ± 0.31^{c}	58.63 ± 0.51^{b}	55.00 ± 1.00^{b}	157.33 ± 0.58^{c}	156.67 ± 1.53^{c}
600 mg/kg b.wt.	83.73 ± 0.45^{d}	62.13 ± 1.17^{c}	59.00 ± 2.00^{c}	163.67 ± 2.08^{d}	160.67 ± 1.53^{d}
800 mg/kg b.wt.	88.47 ± 0.53^{e}	66.53 ± 3.27^{d}	65.33 ± 2.08^{d}	166.67 ± 0.58^{e}	166.33 ± 1.51^{d}

All data are presented as mean ± SEM. Different superscripts down the column indicate that they are significantly different at (p<0.05), n=5

Table 3 showed the serum kidney indices of rats treated with *D. microcarpum* aqueous leaf extract. The serum urea levels of the groups that received extract showed a significant decrease (p<0.05) as the dose of the extract decreases and when compared with the control group. However, the group the received the lowest extract (200 mg/kg b.wt) was not significantly different (p<005) when compared with the control group. Creatinine levels also showed a significant decrease (p<0.05) when compared with the control group. $H^{+}CO_3$ significantly increased (p<005) with increase in dose of the extract and when compared with the control group. Significant increase (p<0.05) was observed in the serum levels of Cl^{-} as dose of the extract increases and when compared with the extract. The serum levels of Na^{+} increased significantly (p<0.05) with increase in dose of the extract and when compared with the control group. Significant increase (p<0.05) was observed in the serum K+ levels as dose of the extract increases and when compared with the control group.

Table 3. Serum kidney indices of rats administered aqueous leaf extract of *D. microcarpum*

Group	Urea (mmol/L)	Creatinine (mmol/L)	H^+CO_3 (mmol/L)	Cl^- (mmol/L)	Na^+ (mmol/L)	K^+ (mmol/L)
Control	19.20 ± 0.75^b	1.13 ± 0.01^d	24.67 ± 0.88^a	95.60 ± 0.70^a	139.67 ± 0.58^a	3.80 ± 0.10^a
200 mg/kg b.wt.	18.83 ± 0.95^b	0.57 ± 0.06^a	25.33 ± 0.58^a	98.87 ± 0.32^b	142.00 ± 1.00^b	4.40 ± 2.00^b
400 mg/kg b.wt.	17.80 ± 0.40^{ab}	0.67 ± 0.06^{ab}	25.33 ± 0.58^a	99.83 ± 0.31^b	142.67 ± 0.58^b	4.57 ± 0.15^b
600 mg/kg b.wt.	17.20 ± 1.25^a	0.77 ± 0.06^{bc}	26.67 ± 0.58^b	102.46 ± 2.05^c	144.33 ± 0.58^c	4.90 ± 0.17^c
800 mg/kg b.wt.	17.07 ± 1.25^a	0.83 ± 0.06^c	27.00 ± 1.00^c	106.83 ± 0.31^d	145.38 ± 0.58^c	5.00 ± 0.10^c

All data are presented as mean ± SEM. Different superscripts down the column indicate that they are significantly different at ($p<0.05$), n=5.

DISCUSSION

The liver maintains homeostasis in living systems. It is involved in biochemical pathways necessary for growth and for fighting against diseases (Ward and Daly, 1999). Aspartate aminotransferase (AST) and alkaline phosphatase (ALT) indicate cellular leakage and loss of the functional integrity of the hepatocyte membrane architecture (Fki *et al.*, 2020), and these two enzymes are considered suitable markers for liver inflammation and necrosis (Fki *et al.*, 2020). The current results disclose that D. microcarpum aqueous leaf extract altered the membrane architecture by damaging the hepatocytes, as the levels of ALT and AST escalated significantly in a dose-dependent manner. Alkaline phosphatase (ALP) is a hydrolytic enzyme responsible for the removal of phosphate groups from many types of molecules, including nucleotides and proteins, and is particularly concentrated in the liver, bile duct, kidney, bone, and placenta (Chukwudoruo *et al.*, 2021). An increase in ALP indicates an obstruction of the bile duct, consequently affecting the liver. An increase in ALP may also result from celiac disease (Tamas *et al.*, 2002). The concentrations of total protein and albumin may indicate the state of the liver and the type of damage (Yakubu *et al.*, 2005). The study also suggests that toxic metabolites in D. microcarpum aqueous leaf extract may be responsible for the significantly high values of total protein and albumin in rats administered with the extract. The albumin and total protein concentrations

significantly increased ($p<0.05$), indicating conditions that cause overproduction of proteins.

The functional capacity of the kidney can be assessed through dye excretion tests, clearance tests, concentration and dilution tests, and methods for examining blood concentrations of excretory and electrolyte constituents (Yakubu *et al.*, 2003). Furthermore, renal function tests are necessary either to demonstrate the presence or absence of active lesions in the kidney or to evaluate the normal functional capacity of various parts of the functioning unit (Talwar *et al.*, 2002). The functional capacity was compromised in the nephron, with a significant increase ($p<0.05$) in Na^+ concentration, likely resulting from excessive loss of the Na^+ pool from body fluids due to the toxic effects of the aqueous leaf extract of *D. microcarpum*. This is corroborated by the significant decrease in Cl^-. Potassium ions play a crucial role in the propagation of nerve impulses along nerve cells and their transmission to receptor cells. The sodium pump maintains the intracellular K^+ concentration against the extracellular K^+ concentration (Burtis and Ashwood, 1999). The hyperkalemia observed suggests a possible adverse effect on the pump that regulates its extracellular concentration due to the renal impairment caused by the extract. Urea is the primary nitrogen-containing metabolic product of protein catabolism. The significant reduction in serum urea concentration throughout the experimental period may be attributed to hindered urea cycle function, which leads to reduced production of this metabolic product. This is particularly evident in the significant reduction observed in creatinine, another product of protein metabolism. Thus, these findings indicate an abnormality in the physiological excretion of urea caused by a non-renal factor. Additionally, since urea synthesis converts toxic ammonia into nontoxic urea, defects in urea synthesis, as observed in this study, may result in ammonia intoxication. In this study, there was a significant increase ($p<0.05$) in the rats treated with higher doses of the plant extract. An increase in serum bicarbonate level within the normal range was associated with a reduced risk of dialysis initiation among the late-stage CKD population (Shah *et al.*, 2009; Raphael *et al.*, 2011). This may suggest reduced waste product removal and excess fluid from the blood due to impaired kidney function resulting from the extract.

CONCLUSION

In conclusion, this study confirms that aqueous leaf extract of *D. microcarpum* exhibits some levels of toxicity, posing significant risks to human health and the environment. Further research is necessary to understand the full scope of its toxicological profile and develop strategies for safe handling and mitigation. The findings of this study underscore the importance of cautious handling and responsible management of *D. microcarpum* to prevent potential harm.

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