Research Article

Analgesic and anti-inflammatory activities of the leaf extracts of *Detarium microcarpum* Guill & Perr (Fabaceae)

Jane David[1*], Jacob A. Kolawole[2], Taiwo E. Alemika[2], Francis M. Agwom[2], Ukpe Ajima[2]

[1]*Department of Medical Biotechnology, National Biotechnology Development Agency, Abuja, Nigeria*

[2]*Department of Pharmaceutical & Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria*

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Abstract

Background: *Detarium microcarpum* is an important leguminous plant that is used for both nutritional and medicinal purposes across different regions of Africa. **Objectives:** The present study is aimed at evaluating the analgesic and anti-inflammatory activities of different leaf extracts of the plant so as to provide a scientific basis for its use in traditional medicine. **Material and methods:** The leaves of the plant were extracted successively using n-hexane, ethylacetate, methanol and water. The extracts were all screened to determine their phytochemical constituents. Furthermore, the hot plate and acetic acid induced writhing test were used to evaluate the analgesic activity of the extracts. For determination of the acute anti-inflammatory activity of the extracts, fresh egg white induced-paw oedema model in wistar albino rats was used while the formaldehyde induced-paw oedema method was used to evaluate the chronic anti-inflammatory activity. **Results:** Phytochemical investigation revealed that the leaf extracts contain saponins, tannins, flavonoids, cardiac glycosides steroids and terpenes with the ethylacetate extract found to be richest in phytochemicals. The extracts of *D. microcarpum* showed dose dependent analgesic activity in the study with the methanol and ethylacetate extracts of the plant having the highest activity in inhibiting acute and chronic inflammation respectively as compared to the reference analgesic diclofenac. **Conclusion:** It can therefore be concluded that there is a scientific rationale to explain the use of the plant in ethnomedicine for the treatment of pain and inflammatory conditions and the leaves of the plant can be further explored in the search for new drugs for the treatment of these conditions.

***Keywords*:** *Detarium microcarpum*, analgesic, anti-inflammatory, hot plate assay, paw oedema

Introduction

Pain has been defined as "a distressing experience associated with actual or potential tissue damage with sensory, emotional, cognitive, and social components" (Williams and Craig, 2016). Pain can be classified as acute or chronic based on its duration; neuropathic or nociceptive depending on its origin and may also be graded as mild, moderate or severe depending on its intensity (Leyva et al., 2019). Nonsteroidal anti-inflammatory drugs (NSAIDs) are used in the management of acute and chronic pain of varying causes in addition to their use in the management of inflammatory conditions such as arthritis (Wong, 2019). They are however known to be associated with gastrointestinal and renal toxicities (Payne, 2000). They are also known to have potential for interaction with other drugs being used for treating co-morbid diseases such as asthma. Steroidal anti-inflammatory drugs like corticosteroids can also be used to treat inflammation but they have limitations such as immune suppression, osteoporosis and hyperglycemia among others (Manson et al., 2009). This necessitates the need to search for newer and safer alternatives from natural sources.

Detarium microcarpum is a leguminous tree which is found mostly in the forest and dry savannah regions of West and Central Africa. The tree possesses both nutritional and medicinal value to the indigenous people of these areas. It has been documented as a major African medicinal plant (Iwu, 2014) and traditional medicine practitioners have used different parts of the plant for centuries in the management of different diseases. The stem bark, seeds, leaves and roots of *D. microcarpum* are widely used in herbal medicine in Nigeria and other parts of Africa. They are prepared as infusions or decoctions to treat venereal diseases, urogenital infections, hemorrhoids, rheumatism, stomachache, intestinal worms and diarrhea (Abreu and Relva, 2002). *D. microcarpum* has also been reportedly used for the treatment

Address for Corresponding Author:
Jane David
Department of Medical Biotechnology, National Biotechnology Development Agency, Abuja, Nigeria
Email: kanjane41@yahoo.com

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of inflammatory conditions in traditional medicine (Burkill, 2004; Mariod et al., 2019). The goal of this study was therefore to investigate the analgesic and anti-inflammatory properties of various extracts of *D. microcarpum* leaves using several experimental animal models with the aim of validating some of the ethnomedicinal claims associated with the plant.

Materials and methods

Plant Collection and Identification

Leaves of *D. microcarpum* were collected from Jos – Plateau State, Nigeria in April 2018 and identified. The identity of the plant was authenticated at Federal College of Forestry, Jos and a specimen with Voucher No. FHJ 234 was deposited in the Faculty of Pharmaceutical Sciences herbarium of University of Jos. Fully expanded leaves were shade dried in the open air and subsequently pulverized.

Extraction

A 1000-gram quantity of the air-dried leaves was milled to powder using a Hammer Mill (grinder). Successive extraction was done using the cold maceration method with n-hexane, ethylacetate, methanol and water. The extracts obtained were collected and concentrated using a rotary evaporator at 40ºC. The concentrated extracts were then weighed and were subjected to preliminary phytochemical screening and biological studies.

Phytochemical screening

Preliminary phytochemical screening was carried out on the different extracts for the presence of secondary metabolites using standard methods and procedures (Sofowora, 2008).

Animals

Wistar albino rats of both sexes weighing between 20 – 30 grams were sourced from the animal house unit of the Pharmacology Department of the University of Jos. The animals were maintained at room temperature and 12-hour light/dark cycle during the experimentation. They were fasted for 8 hours before each test and given water *ad libitum*. Ethical approval for the study was obtained from the Institutional animal care and use committee of the Pharmacology Department, University of Jos and it was conducted according to approved guidelines and ethical principles of animal research.

Hot plate test for analgesic activity

Male and Female wistar albino rats were assigned into groups (n = 5/group) and treated with the various extracts at doses of 250, 500 and 1000 mg/kg body weight, 5 ml/kg of normal saline (control) and 30 mg/kg Pentazocine sub-cutaneously (standard) The reaction times of these rats were measured 1 hour prior to the treatment and 30 minutes after the treatment using hot plate techniques as described (Langerman et al., 1995). In the hot plate test, the rat was placed in a hot plate analgesia meter at 50 °C and the time taken to lick the hind paw or to jump was recorded. Rats showing a pre-treatment reaction time greater than 15 seconds (s) in the hot plate test were not used in the experiment. A cut off time of 25 seconds was set to avoid tissue damage.

Acetic acid-induced writhing test analgesic assay

Acetic acid induced writhing method (Saifuzzaman et al., 2013) was adopted for this assay. Twenty-five rats of either sex were divided into five groups of five rats each. Groups 1, 2 and 3 were treated with extract at doses of 250, 500 and 1000 mg/kg body weight intraperitoneally (i.p.) respectively. Group 4 was treated with diclofenac 20 mg/kg body weight intraperitoneally while group 5 (control) received normal saline 5 ml/kg body weight intraperitoneally. Thirty minutes post treatment, mice in all groups were administered 0.6% freshly prepared acetic acid solution (10 ml/kg i.p.) and the number of abdominal constrictions was counted for each animal five minutes after administration for the next 10 minutes.

Acute Inflammation: Fresh Egg White-induced paw oedema model

Acute inflammation was achieved using the egg white induced oedema model (Karthikeyan and Deepa, 2011). The rats were starved overnight after which they were divided into five groups (A-E) of 5 animals each (n=5). All the rats in all the five groups (A-E) were injected with 0.05ml of fresh undiluted egg white at right hand paw 1 hour after the drug treatment.

Group A (control): [untreated 10 ml/kg saline p.o + 0.05ml of fresh egg white]

Group B (standard): [diclofenac 25 mg/kg p.o + 0.05ml of fresh egg white]

Group C (Test 1): [250 mg/kg extract + 0.05ml of fresh egg white]

Group D (Test 2): [500 mg/kg extract + 0.05ml of fresh egg white]

Group E (Test 3): [1000 mg/kg extract + 0.05ml of fresh egg white]

a) The linear circumference of the paw was measured after 0 hour and 3 hours of fresh egg white injection using a digital Vanier caliper.

The percentage inhibition was calculated according to the formula:

Percentage inhibition = {1 – [(C1-C0) test / (C1-C0) control]} x 100

C0 = Mean paw size at 0 h after fresh egg white

C1 = Mean paw size at 3 h after fresh egg white

Chronic Inflammation: Formaldehyde-induced paw oedema model

Male and female rats (n = 5/group) were treated with 250, 500 and 1000 mg/kg/day of extracts, 25 mg/kg/day Diclofenac and 5 ml/kg/day of normal saline for 6 consecutive days. After 1 hour on days 1 and 3 of treatment, these rats were injected with 0.1 ml of 2% formaldehyde into the foot pad of left hind paw (Akinnawo et al., 2017). Paw oedema was measured 1 hour before formaldehyde injection and at 4 hours after the injection on day 1 and every day at 1 hour after the treatment for 6 consecutive days.

Statistical analysis

All results were expressed as mean ± SEM. The significance of differences between treated groups was determined using one-way analysis of variance (ANOVA) followed by Dunnets' post hoc test. P-values < 0.05 were considered significant. Statistical Package for Social Scientist (SPSS) Version 22.0 software was used for all statistical analysis.

Results and discussion

The results of phytochemical screening of the extracts of *D. microcarpum* are summarized in table 1. It reveals the presence of carbohydrates in all the extracts while flavonoid and tannins were found in only the ethylacetate, methanol and aqueous extracts. Steroids were found in the n-hexane, ethylacetate and methanol extracts but cardiac glycosides were found in only the ethylacetate and methanol extracts. Alkaloids and anthraquinones were absent in all the extracts. However, saponins are present only in the aqueous extract while terpenes are present in only the ethylacetate extract of the leaves.

The results of evaluation of the analgesic activity of the four extracts of *D. microcarpum* using the hot plate method in wistar albino rats after 30 minutes of treatment are presented in table 2. It can be seen that all the extracts enhanced the interval of heat response in the rats and the analgesic effect increased with the increasing dosage from 250 mg/kg body weight but reached a peak at 500 mg/kg before a subsequent decline when the dose increased to 100 mg/kg.

The results of assessment of the analgesic activity of the

Table 1. Results of the phytochemical screening of the various extracts of *D. Microcarpum* leaves using different solvents

Chemical constituents	n-hexane	Ethyl Acetate	Methanol	Aqueous
Alkaloids	-	-	-	-
Saponins		-	-	+
Tannins	-	++	++	+
Flavonoids		+++	+++	+
Carbohydrates	+	+	+++	++
Steroids	++	+	++	-
Terpenoids	-	++	-	-
Anthraquinones	-	-	-	-
Cardiac glycosides	-	+	+	-

Key: - = Absent; + = Slightly present; ++ = Moderately Present; +++ = Significantly present

Table 2. Analgesic activity of extracts of *D. microcarpum* using wistar albino rats (Hot plate method) after 30 minutes of treatment

Treatment groups	Extract dose 250 mg/kg		Extract dose 500 mg/kg		Extract dose 1000 mg/kg	
	Reaction time (s)	%Inhibition	Reaction time (s)	%Inhibition	Reaction time (s)	%Inhibition
Normal Saline	14.57±1.72	-	14.57±1.72	-	14.57±1.72	-
n hexane	18.45±2.20	26.63	19.37±4.69	32.94	18.12±2.40	24.36
Methanol	24.00±1.38	66.78	38.77±8.13[a]	88.50	66.65±20.38[ab]	87.60
Ethylacetate	19.37±4.34	44.17	17.87±1.25[b]	22.81	17.40±1.83	8.87
Aqueous	24.30±6.24	32.94	33.07±3.55[a]	22.64	35.97±13.15	19.42
Pentazocine	31.55±3.28	66.43	31.55±3.28	66.43	31.55±3.28	66.43

a= significantly different when compared with standard (30 mg/kg pentazocine); b= significantly different when compared with Normal saline at ($p<0.05$); Values are mean ± SEM; n=5

four extracts using the acetic acid induced writhing method in wistar albino rats are presented in table 3. It was observed that all the extracts showed considerable decrease in the number of writhing in a dose dependent manner. Tables 4, 5, 6 and 7 show the results of the acute inflammation studies using the fresh egg white induced paw oedema for the n-hexane, ethylacetate, methanol and aqueous leaves extract of the plant respectively. Similarly, tables 8, 9, 10 and 11 show the results of the chronic inflammation studies using the formaldehyde induced paw oedema for the n-hexane, ethylacetate, methanol and aqueous leaf extract of *D. microcarpum* respectively. The tables show the paw oedema (mL) measured at different time intervals with the aid of a plethysmomether.

The study revealed that the leaves of *D. microcarpum* contain an array of phytochemicals which have potential for the treatment of some painful and inflammatory disease conditions. Phytochemical investigations revealed that the various leaf extracts contain saponins, tannins, flavonoids, cardiac glycosides, steroids and terpenes. This agrees with the observation of earlier authors who found similar constituents in the leaf extracts of the plant (Iwu, 2014; Yaro et al., 2017). However, alkaloids which was not detected in this study had earlier been reported by some of these authors. This suggests a possible variation in phytochemical constituents of the plants which can arise due to several factors such as climate, geographical location, soil nutrients, stress and time of plant harvest (Gulzar, 2017).

The hot plate method is a model used for assessing the mechanism of central analgesia which involves the activation of opioid receptors in the central nervous system, increasing the threshold for pain and altering the physiological response to pain (Hijazi et al., 2017). The hot plate assay revealed that the extracts have moderate analgesic activity as compared to the standard drug Pentazocine. It was observed that all the extracts prolonged the latency of heat response in the rats and the analgesic effect increased with increasing dosage from 250 mg/kg body weight but reached a peak at 500 mg/kg before a subsequent decline when the dose increased to 1000 mg/kg. The results as shown in table 2 also reveal that the activity of the methanolic extract obtained in this study is in good agreement with a previous study (Yaro et al., 2017).

Table 3. Analgesic activity of extracts of *D. microcarpum* using wistar albino rats (Acetic acid induced writhing model)

Treatment groups	Extracts dose, 250mg/kg		Extracts dose, 500mg/kg		Extracts dose, 1000mg/kg	
	Number of writhing movements	%Inhibition	Number of writhing movements	%Inhibition	Number of writhing movements	%Inhibition
Ethylacetate	33.20±1.71^{ab}	17.41	28.20±0.73^{ab}	29.83	22.00±2.77^{ab}	50.25
n-Hexane	37.60±1.12^{a}	6.47	32.60±1.07^{ab}	18.91	28.80±0.58^{ab}	28.36
Aqueous	36.20±0.66^{a}	9.95	30.40±0.87^{ab}	24.38	24.60±0.97^{ab}	38.81
Methanol	31.80±4.23^{ab}	21.89	24.20±2.03^{ab}	37.31	18.20±1.59^{b}	54.73
Normal saline	40.20±0.86	-	40.20±0.86	-	40.20±0.86	-
Diclofenac	16.80±1.15	58.21	16.80±1.5	58.21	16.80±1.5	58.21

a= significantly different when compared with standard (diclofenac 20 mg/kg); b= significantly different when compared with normal saline (5 ml/kg) at ($p<0.05$); Values are mean ± SEM; n=5

Table 4. Acute inflammatory studies of n-hexane extract of *D. microcarpum* using wistar albino rats (Fresh egg white induced paw oedema method)

Treatment groups	Time duration						
	0min	30 mins	60mins	90 mins	120 mins	150 mins	180 mins
Control	5.31±0.22	7.07±0.34	6.89±0.29	6.56±0.33	6.29±0.29	5.92±0.27	5.97±0.30
250 mg/kg	5.40±0.30	6.60±0.25	4.94±0.48	7.14±0.34^{b}	7.23±0.23^{b}	6.56±0.26	6.05±0.43
500 mg/kg	4.84±0.11	5.40±0.28^{a}	6.85±0.51	6.40±0.38	6.27±0.48	6.02±0.36	5.69±0.16
1000 mg/kg	4.77±0.46	6.06±0.14	6.97±0.38	7.59±0.30^{b}	6.96±0.0.09^{b}	6.44±0.29	5.76±0.16
standard (25 mg/kg diclofenac)	4.92±0.10	6.53±0.44	5.98±0.36	5.95±0.31	5.29±0.38	5.22±0.34	5.06±0.28

a= significantly different when compared with standard (25 mg/kg diclofenac); b= significantly different when compared with Normal saline at ($p<0.05$); Values are mean ± SEM; n=5

Acetic acid induced writhing model is used to evaluate peripheral analgesic activity. This is thought to involve local peritoneal receptors, whose stimulation have been linked to increased levels of prostaglandins, histamine, bradykinins and other mediators of inflammation, in addition to lipoxygenase products that enhance inflammatory pain via increases in capillary permeability (Sireeratawong et al., 2012). The methanol and ethyl acetate leaf extracts of *D. microcarpum* showed significant analgesic activity which was comparable to the standard drug (diclofenac 20 mg/kg) as shown in table 3. Drugs that act peripherally inhibit the pain generation impulse at the chemoreceptor level (Shreedhara et al., 2009)

Table 5. Acute inflammatory studies of Ethyl acetate extract of *D. microcarpum* using wistar albino rats (Fresh egg white induced paw oedema method)

Treatment groups	Time duration						
	0 min	30 min	60 min	90 min	120 min	150 min	180 min
Control	5.31±0.22	7.07±0.34	6.89±0.29	6.56±0.33	6.29±0.29	5.92±0.27	5.97±0.30
250 mg/kg	5.29±0.21	6.62±0.14	6.71±0.17	6.58±0.10	6.22±0.14^{b}	5.99±0.06^{b}	5.57±0.12
500 mg/kg	4.90±0.11	6.52±0.19	6.61±0.12	6.16±0.15	5.94±0.15	5.31±0.13	4.80±0.18^{a}
1000 mg/kg	4.87±0.18	5.54±0.40^{ab}	5.25±0.26^{a}	5.09±0.24^{ab}	4.73±0.27^{a}	4.44±0.33^{ab}	4.16±0.28^{ab}
Standard (25 mg/kg diclofenac)	4.92±0.10	6.53±0.44	5.98±0.36	5.95±0.31	5.29±0.38	5.22±0.34	5.06±0.28

a= significantly different when compared with standard (25 mg/kg diclofenac); b= significantly different when compared with Normal saline at ($p<0.05$); Values are mean ± SEM; n=5

Table 6. Acute inflammatory studies of methanolic extract of *D. microcarpum* using wistar albino rats (Fresh egg white induced paw oedema method)

Treatment	Time duration						
	0 mins	30 mins	60 mins	90 mins	120 mins	150 mins	180 mins
Control	5.31±0.22	7.07±0.34	6.89±0.29	6.56±0.33	6.29±0.29	5.92±0.27	5.97±0.30
250 mg/kg	3.71±0.14^{ab}	4.61±0.24^{ab}	4.72±0.31^{a}	4.72±0.29^{ab}	4.73±0.34^{a}	4.57±0.31^{a}	4.16±0.15^{a}
500 mg/kg	3.51±0.15^{ab}	4.63±0.28^{a}	4.97±0.35^{a}	5.17±0.47^{a}	4.75±0.41^{a}	4.60±0.49^{a}	4.16±0.36^{a}
1000 mg/kg	3.67±0.12^{ab}	3.86±0.10^{ab}	3.82±0.15^{a}	3.74±0.06^{ab}	4.02±0.11^{ab}	3.55±0.22^{ab}	3.75±0.28^{ab}
standard (25 mg/kg diclofenac)	4.92±0.10	6.53±0.44	5.98±0.36	5.95±0.31	5.29±0.38	5.22±0.34	5.06±0.28

a= significantly different when compared with standard (25 mg/kg diclofenac); b= significantly different when compared with Normal saline at ($p<0.05$); Values are mean ± SEM; n=5

Table 7. Acute inflammatory studies of aqueous extract of *D. microcarpum* using wistar albino rats (Fresh egg white induced paw oedema method)

Treatment groups	Time duration						
	0 min	30 min	60 min	90 min	120 min	150 min	180 min
Control	5.31±0.22	7.07±0.34	6.89±0.29	6.56±0.33	6.29±0.29	5.92±0.27	5.97±0.30
250 mg/kg	3.95±0.16^{ab}	5.60±0.27^{a}	5.74±0.17^{a}	5.60±0.15	5.33±0.15	5.30±0.28	4.81±0.46^{a}
500 mg/kg	3.82±0.12^{ab}	4.98±0.07^{ab}	5.24±0.17^{a}	5.26±0.25^{a}	4.93±0.30^{a}	5.03±0.15	4.92±0.19^{a}
1000 mg/kg	3.70±0.20^{ab}	5.03±0.48^{ba}	4.81±0.33^{ab}	4.90±0.41^{ab}	4.63±0.33^{a}	4.88±0.34^{a}	4.40±0.09^{a}
standard (25 mg/kg diclofenac)	4.92±0.10	6.53±0.44	5.98±0.36	5.95±0.31	5.29±0.38	5.22±0.34	5.06±0.28

a= significantly different when compared with standard (25mg/kg diclofenac); b= significantly different when compared with Normal saline at ($p<0.05$); Values are mean ± SEM; n=5

and it is likely that these extracts acted at this level. On the other hand, the n-hexane and aqueous extracts were only weakly active in the assay and this may be linked to the low level of phytochemicals present in them as can be seen in the result of the phytochemical screening.

Acute inflammation is reported to have an early phase that typically last one to two hours and is mainly mediated by histamine and serotonin while the latter phase is mainly mediated by bradykinins, prostaglandins. The results of the egg white induced paw oedema that was used to assess the ability of the extracts to inhibit acute inflammation revealed that they possess dose dependent anti-inflammatory activity. The methanol extract exhibited the strongest anti-inflammatory effect of all the extracts as compared with

Table 8. Chronic Anti-inflammatory studies of n-hexane extract of *D. microcarpum* using wistar albino rats (Formaldehyde induced paw oedema model)

Treatment groups	Time duration						
	1 hour	4hours	Day2	Day3	Day4	Day5	Day6
Control	4.28±0.08	6.89±0.58	7.52±0.60	7.76±0.56	7.75±0.52	7.55±0.42	7.54±0.42
250mg/kg	4.21±0.43	5.32±0.06	5.58±0.18	5.79±0.37	5.42±0.27[a]	5.22±0.04[a]	5.16±0.51[a]
500 mg/kg	4.70±0.45	4.86±0.07	6.07±0.24	5.79±0.16	5.35±0.20[a]	4.91±0.35[a]	4.47±0.54[a]
1000 mg/kg	4.48±0.16	5.29±0.20	6.11±0.38	6.42±0.23	5.27±0.12[a]	4.98±0.10[a]	4.60±0.29[a]
standard (25 mg/kg diclofenac)	5.55±0.57	6.26±0.71	6.00±0.54	6.30±0.70	5.63±0.61	5.29±0.59	4.91±0.58

a= significantly different when compared with standard (25 mg/kg diclofenac); b= significantly different when compared with normal saline at ($p<0.05$); Values are mean ± SEM; n=5

Table 9. Chronic anti-inflammatory studies of ethylacetate extract of *D. microcarpum* using wistar albino rats (Formaldehyde induced paw oedema model)

Treatment groups	Time duration						
	1 hour	4hours	Day2	Day3	Day4	Day5	Day6
Control	4.28±0.08	6.89±0.58	7.52±0.60	7.76±0.56	7.75±0.52	7.55±0.42	7.54±0.42
250 mg/kg	4.62±0.38	5.33±0.38	6.45±0.38	5.83±0.16	6.99±0.27	7.01±0.70	6.60±0.50[b]
500 mg/kg	5.13±0.10	5.17±0.10	6.00±0.63	6.75±0.05	6.41±0.08	6.35±0.68	5.88±0.57[a]
1000 mg/kg	5.08±0.39	4.84±0.15	5.80±0.35	5.90±0.57	5.51±0.37	4.86±0.32	4.71±0.35[a]
standard (25 mg/kg diclofenac)	5.55±0.57	6.26±0.71	6.00±0.54	6.30±0.70	5.63±0.61	5.29±0.59	4.91±0.58

a= significantly different when compared with standard (25 mg/kg diclofenac); b= significantly different when compared with normal saline at ($p<0.05$); Values are mean ± SEM; n=5

Table 10. Chronic anti-inflammatory studies of methanolic extract of *D. microcarpum* using wistar albino rats (Formaldehyde induced paw oedema model)

Treatment groups	Time duration						
	1 hour	4hours	Day2	Day3	Day4	Day5	Day6
Control	4.28±0.08	6.89±0.58	7.52±0.60	7.76±0.56	7.75±0.52	7.55±0.42	7.54±0.42
250 mg/kg	4.06±0.58[b]	4.36±0.25[b]	5.83±0.16	6.49±0.27	6.40±0.28	7.12±0.47[b]	7.25±0.72[b]
500 mg/kg	4.48±0.15	5.25±0.28[a]	6.75±0.05	5.73±0.26	5.81±0.36[a]	5.77±0.29[a]	5.55±0.22[a]
1000 mg/kg	3.89±0.25[b]	5.67±0.28[b]	6.78±0.13	6.29±0.33	5.40±0.46[a]	5.03±0.65[ab]	5.10±0.50[a]
standard (25 mg/kg diclofenac)	5.55±0.57	6.26±0.71	6.00±0.54	6.30±0.70	5.63±0.61	5.29±0.59	4.91±0.58

Table 11. Chronic anti-inflammatory studies of aqueous extract of *D. microcarpum* using wistar albino rats (Formaldehyde induced paw oedema model)

Treatment groups	Time duration						
	1 hour	4 hours	Day 2	Day 3	Day 4	Day 5	Day 6
Control	4.28±0.08	6.89±0.58	7.52±0.60	7.76±0.56	7.75±0.52	7.55±0.42	7.54±0.42
250 mg/kg	4.30±0.12[b]	5.56±0.27	7.16±0.11	6.79±0.25	6.86±0.28	6.14±0.37	5.75±0.50[a]
500 mg/kg	4.77±0.22	5.36±0.74	7.36±0.19	6.81±0.07	6.13±0.44	5.36±0.39[a]	5.29±0.52[a]
1000 mg/kg	5.50±0.20[a]	5.55±0.67	7.25±1.22	7.36±0.61	6.71±0.56	6.76±0.29[b]	6.63±0.37[b]
standard (25 mg/kg diclofenac)	5.55±0.57	6.26±0.71	6.00±0.54	6.30±0.70	5.63±0.61	5.29±0.59	4.91±0.58

a= significantly different when compared with standard (25 mg/kg diclofenac); b= significantly different when compared with control at ($p<0.05$); Values are mean ± SEM; n=5

diclofenac (25 mg/kg). A recent study on *D. microcarpum* leaf extract using the egg white and formalin induced inflammation model also strongly affirms the potent anti-inflammatory effect of the methanolic leaf extract of the plant (Odoh and Ene, 2020).

The formaldehyde induced paw oedema model was used to evaluate the effects of the extract on chronic inflammation and it was observed that the extracts significantly prevented the formation of inflammatory oedema due to challenge with formaldehyde. Specifically, the ethylacetate extract was found to actively prevent inflammation through day four to six, indicating that it is highly effective against the establishment of chronic inflammation. This could be attributed to ability of the extract to inhibit prostaglandin synthesis which plays key roles in countering the establishment of chronic inflammation (Lu et al., 2007). A wide-ranging review of existing literature revealed that no other study has reported similar results as obtained with the ethylacetate leaf extract of *D. microcarpum*. This notable activity may be linked with some of the plant secondary metabolites present in the ethyl acetate leaf extract of the plant which was found to be rich in flavonoids, tannins and terpenes. These phytochemicals have been shown to possess significant anti-inflammatory and analgesic activities (Bittar et al., 2000; Li et al., 2003; Yadav et al., 2010).

Conclusion

The results of the study have demonstrated that the leaf extracts of *D. microcarpum* possess analgesic and anti-inflammatory activities. The results also provide a justification for the use of this plant in managing pain and inflammatory conditions in traditional medicine. Additional research through bioassay guided fractionation is necessary to isolate the active principles responsible for its anti-inflammatory and antinociceptive activities.

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