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Anti-Inflammatory and Analgesic Effect of Detarium Microcarpum (Guill. and Perrs.) Stem Bark Methanol Extract in Rats and Mice

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Abstract— *Detarium microcarpum is a commonly used medicinal plant in northern Nigeria for the management of pain and inflammatory conditions. In this study the methanol stem bark extract of D. microcarpum was evaluated for acute toxicity, analgesic and anti-inflammatory effect. Acute toxicity was performed using Lorke method. The analgesic activity was evaluated using acetic acid induced- writhing and hot plate tests in mice and the anti-inflammatory activity was evaluated using carrageenan-induced rat paw oedema model. The intraperitonial median lethal dose (LD50) of the stem bark extract was 471.2 mg/kg body weight in mice and orally greater than or equal to (≥) 5000 mg/kg body weight in rats. The extract dose-dependently inhibited acetic acid-induced writhing in mice and prolonged the latency of thermally-induced pain. In the carrageenan-induced inflammation the methanol extract of D. microcarpum reduced the mean diameter of the rat paw when compared with the normal saline treated group. The study indicates that D. microcarpum has significant analgesic and anti-inflammatory properties. This corresponds to the ethnomedicinal claim for the usage in pain and inflammation relief.*

Keywords— *Analgesia; Anti-inflammation; Detarium microcarpum; carrageenan; ethnomedicinal.*

I. INTRODUCTION

Pain is a sensory and emotional experience characterized with potential tissue damage. Pain can either be neuropathic, inflammatory, nociceptive and functional. Management of pain has to take this fact into this consideration (Rajagopal, 2006). Inflammation is the fundamental defensive reaction of the body to an inversion of pathogens or injury it involves a complex array of enzyme reaction; mediators release, extravasations, cell migration, tissue breakdown and repair (Herrero *et al.*, 1997). The sings of inflammation such as pain, redness, swelling and loss of functions are produce by inflammatory agents such as nitric oxide, prostaglandins, bradykinin, serotonin, leukotrienes and histamine (Ahmadiani *et al.*, 2000). Uncontrolled and persistent inflammation contributes to the progression of many chronic disease such as multiple sclerosis, rheumatoid arthritis, atherosclerosis, psoriasis and inflammatory bowel disease (Vittalrao *et al.*, 2011). Though inflammation is a defense mechanism, the complex events and mediators involved in inflammatory reactions can induce or aggravate many reactions (Dalgleish and O'Byrne, 2002).

Detarium microcarpum Guill and Perr, known as Tallo tree belong to the family Caesalpiniaceae D. microcarpum is a small tree, reaching 10 m high, with a dense rounded crown in dry areas and it reach up to 25 m high in wetlands. D. microcarpum stem bark extract is locally used for the treatment of diarrhea, amoebiasis, gonorrhea, hemorrhoids and rheumatism (Mariod *et al.*, 2009). They are prepared as infusions or decoctions to treat rheumatism, venereal disease, urogenital infections, stomach ache, intestinal worms and diarrhea including dysentery. The leaves, barks and fruits are used to treatment of ailments such as tuberculosis and itching (Igwe and Friday, 2017; Mariod, *et al.*, 2009).

The phytochemical analysis of the stem bark extract contains some appreciable amount of cardiac glycosides, tannins, saponins, alkaloids, polyphenols and volatile oils. Its antifungal and acetyl cholinesterase inhibitory activities have been reported (Adamu *et al.*, 2006). The methanol and aqueous stem back extracts are reported to possess antimicrobial activity and reduced liver damage induce by mycotoxins (Hamza et al., 2014; Mohammed et al., 2016).In order to search for newer remedy for analgesia and inflammation, this study aimed to investigate the analgesic and anti-inflammatory effects of stem bark extract of Detarium microcarpum in rats and mice.

II. MATERIALS AND METHODS

2.1 Plant Materials

Stem bark of the Detarium microcarpum was collected from Tsolonbashi village, Jigawa State, Nigeria. The plant was identified and authenticated with voucher specimen number 0071 in the Herbarium of the Biological sciences Department, Bayero University, Kano.

2.2 Experimental Animals

Swiss albino mice (18-32g) and Wistar rats (80-130g) of either sex were obtained from the Department of Pharmacology, Bayero University, Kano. The animals were allowed free access to standard feed and water ad libitum. Experiments were conducted in accordance with National Institute of Health Guidelines for the care and use of

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Laboratory Animals revised in 1996 (NIH Publication No. 80-23)

2.3 Drugs and Chemicals

Methanol, Carrageenan, and Formalin solution were purchased from Sigma–Aldrich (Steinheim, *Germany*), Acetic acid (BDH Poole, England), Ketoprofen (Lek, pharmaceuticals company, Slovenia), Pentazocine (Fidson).

2.4 Preparation of Extract

The stem bark collected was air dried pulverized using mortar and pestle and sieved. The powdered plant material was macerated in 70% methanol and kept for seven days with occasional stirring. The extract was then sieved using mesh and filtered using whatman filter paper. The filtrate was concentrated to dryness on water bath at 40°C until the solvent was completely evaporated. The resultant powdered extract obtained was stored in a dessicator until required.

2.5 Acute Toxicity Study

2.5.1 Median Lethal Dose (LD50) Determination

The LD_{50} of the extract was determined using Lorke's (1983) method. Animals were deprived of food for 12-16 hours prior to administration of extract. The study was carried out in two phases. In phase one, three groups of three mice per group were used. The extract was administered intraperitoneally (i.p.) in geometrical increasing doses (10 mg/kg, 100 mg/kg and 1000 mg/kg). The treated animals were observed four hours post administration and subsequently for 24 hours for signs of toxicity including death. In the second phase specific doses of (140, 225, 370 and 600 mg/kg) were administered based on the result of the first phase. The LD50 value was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose. The same procedure was employed in determination of oral LD50 values in rats.

2.6 Pharmacological Studies

2.6.1 Analgesic studies

2.6.1.1 Acetic acid-induced writhing test in mice

Acetic acid induced writhing method described by Koster et al (1959) was adopted. Thirty mice of either sex were divided into five groups of six mice each. Groups 1, 2 and 3 were treated with extract at doses of 35, 70 and 140 mg/kg (i.p.) body weights respectively. Group 4 was treated with ketoprofen 10 mg/kg body weight intraperitonially while group 5 (control) received normal saline 10 ml/kg body weight intraperitonially. 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. Percentage of inhibition of abdominal constrictions was calculated using the following formula;

$$Inhibition(\%) = \frac{Mean\,Number\,of\,writhes(control) - Mean\,Number\,of\,writhes(test)}{Mean\,Number\,of\,writhes\,(control)} \times 100 \quad (1)$$

2.6.1.2 Hot plate test in mice

The test was performed using a hot plate maintained at 45±1°C as described by Eddy and Leimback (1953). Mice that showed nociceptive responses within 20s when placed on the hot plate were selected into the study and divided into five groups of six mice each. The first group received normal saline (10 ml/kg) (*i.p.*), the second, third and fourth groups were given 35, 70 and 140 mg/kg of the extract (*i.p.*) respectively, and the last group received pentazocine 10mg/kg (*i.p.*). Thirty minutes later each mouse was placed on the hot plate and the time taken by the mice to respond to the thermal stimulus was recorded. Reaction time was taken as the interval between the time the animal was placed on the hot plate till the moment it began to lick its paws or jump off. Sixty seconds was chosen as the cut off time to avoid tissue damage. Readings were taken for each mouse at time 0 and after every 30 minutes until the 90th minute post treatment.

2.6.2 Anti-inflammatory Studies

2.6.2.1 Carrageenan-induced paw oedema in rats

The method of Winter *et al* (1963) was used. Twenty five adult Wistar rats of either sex were randomly divided into 5 groups of 5 rats each. The first group received normal saline (1 ml/kg) (i.p.), the second, third and fourth groups were given 35, 70 and 140 mg/kg of the extract (i.p.), respectively, and the last group received ketoprofen (10 mg/kg). Thirty minutes later, 0.1 ml of freshly prepared 1% carrageenan suspension was injected into the sub-plantar region of the left hind paw of each rat. Measurements of the paw odema were then taken with a digital vernire caliper at 0, 1, 2, 3 and 4 h after the injection of carrageenan.

2.7 Statistical Analysis

Data obtained were expressed as Mean ± Standard Error of Mean (Mean ± SEM), and presented as tables and plates where appropriate. Data were analyzed using one way ANOVA followed by Dunnett's post hoc test. Values of p< 0.05 were considered statistically significant.

III. Results and Findings

3.1 Median Lethal Dose (LD_{50}) of Methanol Stem Bark Extract of Detarium microcarpum

The intraperitonial median lethal dose (LD_{50}) of methanol stem bark extract of Detarium microcarpum in mice was calculated to be 471.2 mg/kg while the oral LD50 in rats was found to be greater than 5000 mg/kg body weight (Table I).

TABLE I. Median lethal dose of methanol stem bark extract of Detarium microcarpum in mice and rats

Species	Route of administration	LD50 (mg/kg body weight)
Mice	Intraperitonial	471.2
Rats	Oral	>5000

3.2 Analgesic Studies

3.2.1 Acetic acid-induced writhing test in mice

The extract significantly (p≤ 0.05) and dose dependently inhibited acetic acid-induced writhes in mice. The highest inhibition was produced at a dose of 140 mg/kg (82.2%) which was greater than that of the 10 mg/kg ketoprofen (68.9%) standard drug (Table II).

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TABLE II. Effect of methanol stem bark extract of Detarium microcarpum on acetic acid-induced writhing in mice.

Treatment (mg/kg)	Mean No. of writhes ± SEM	% of Inhibition
Nomal saline (10 ml/kg)	22.5 ± 4.03	
Ketoprofen (10	7.00 ± 1.53[a]	68.9
D.M (35)	8.67 ± 1.91[a]	62.2
D.M (70)	7.00 ± 2.59[a]	68.9
D.M (140)	4.00 ± 1.18[b]	82.2

Data were analyzed using one way ANOVA followed by Dunnet post hoc test. Values expressed as Mean ± SEM and percentages, a, b, represent $p< 0.05$, $p < 0.01$, n=6. D.M = Extract of Detarium microcarpum.

3.2.2 Hot plate test in mice

The extract at all doses tested (35, 70 and 140 mg/kg body weight) significantly ($p\leq 0.05$) prolonged latency of pain reaction induced by hot plate. At the highest dose (140 mg/kg) the extract significantly ($p\leq 0.05$) prolonged the pain reaction time up to 90 minutes. Pentazocine (10 mg/kg) the standard drug used, significantly ($p\leq 0.05$) increased reaction time up to 60 minutes (Table III).

TABLE III. Effect of methanol stem bark extract of *Detarium microcarpum* on thermally induced-pain in mice.

Treatment (mg/kg)	Mean reaction time in (s) ± SEM			
	Pre-treatment	30 min	60 min	90 min
Normal saline (10 ml/kg)	2.26± 0.42	2.18±0.18	1.86± 0.18	2.34±0.25
Pentazocine (10)	2.42±0.17	3.70±0.33[a]	3.85±0.53[a]	2.65± 0.42
D.M (35)	2.63± 0.18	3.03± 0.53	2.97±0.28[a]	2.71± 0.29
D.M (70)	3.13± 0.31	3.92±0.57[a]	3.92±.52[b]	3.57± 0.58
D.M (140)	3.30± 0.51	4.19±0.55[a]	4.61±0.41[b]	3.63±0.33[a]

Data were analyzed using one way ANOVA followed by Dunnet post hoc test. Values expressed as Mean ± SEM, a, b, represent $p< 0.05$, $p < 0.01$, n=6/ group, D.M = Extract of Detarium microcarpum.

3.3 Anti-inflammatory Studies

3.3.1 Carrageenan induced paw oedema in rats

Sub planter administration of 1% carrageenan in the normal saline treated group produced local oedema reaching its maximum at 2 hours. The methanol stem back extract at a dose of 35mg/kg also produced oedema reaching maximum after 2 hours. However, the extract at a dose of 70 mg/kg significantly ($p\leq 0.05$) inhibited development of oedema from 2 hours up to 4 hours. At highest dose of 140 mg/kg the extract significantly ($p\leq 0.05$) decreased paw oedema from 1st up to 4th hour. Standard drug ketoprofen (10 mg/kg) also significantly ($p\leq 0.05$) decreased oedema from 1st to 4th hour (Table IV).

TABLE IV. Effect of methanol stem bark extract of Detarium microcarpum on carrageenan induced rat paw oedema.

Treatment (mg/kg)	MEAN PAW DIAMETER (mm)				
	Pre-treatment	1 hr	2 hr	3 hr	4 hr
N/Saline (1 ml/kg)	1.32±0.22	2.12±0.46	2.55±0.40	2.42±4.009	1.87±0.29
Ketoprofen (10)	1.00±0.19	0.70±0.21[a]	0.69±0.12[c]	0.51±0.24[c]	0.61±0.18[c]
D.M (35)	1.38±0.12	1.76±0.27	1.93±0.27	1.74±0.21	1.51±0.25
D.M (70)	1.27±0.12	1.09±0.14	0.96±0.21[a]	1.04±0.20[a]	0.82±0.18[b]
D.M (140)	1.42±0.21	0.79±0.24[a]	0.85±0.21[b]	0.57±0.19[b]	0.74±0.26[b]

Data were analyzed using one way ANOVA followed by Dunnet post hoc test. Values expressed as Mean ± SEM, a, b and c represent $p< 0.05$, $p < 0.01$, $p < 0.001$ respectively, n=5/group, D.M = Extract of Detarium microcarpum.

IV. DISCUSSION

Acute toxicity studies are usually carried out to determine the dose that will cause death or serious toxic manifestations when administered singly or severally at few doses in order to establish dose that should be used in subsequent studies (Haschek and Rousseaux, 2013). The intraperitonial median lethal dose value of the methanol extract of Detarium microcarpum stem bark obtained in mice was found to be 471.2 mg/kg and the oral median lethal dose of the extract was found to be (≥ 5000) in rats this may probably due to high absorption when administered intraperitonially. This shows that the extract was practically toxic when administered intraperitonially and nontoxic when taken orally (Lorke, 1983).

Writhing is explain as a stretch tension to one side, extension of hind legs, contraction of abdomen so that the abdomen of mice touches the floor, or turning of trunk (Mishra *et al.*, 2011). Acetic acid induced abdominal pain is used for the evaluation of peripheral analgesic activity (Gené *et al.*, 1998). The abdominal constriction response is thought to involve in part of local peritoneal receptors; which has been associated with increased peritoneal fluid concentration of PGE2 and PGE2α serotonin, bradykinins, substance P, histamine as well as lipooxygenase products which enhance inflammatory pain by increasing capillary permeability, the release of cytokines such as interleukin Iβ, TNF-α by peritoneal macrophages may also be involved in abdominal constriction following intraperitoneal injection of acetic acid (Lakshman *et al.*, 2006).

The mechanism by which substances inhibit writhing is by inhibition of prostaglandin synthesis or its action on peritoneal receptors (Mishra*, et al.*, 2011). The methanol stem bark extract of Detarium microcarpum inhibited acetic acid induced-writhing at all the doses tested which suggest that the methanol extract stem bark possess peripheral analgesic properties.

Hot plate method as described by Eddy and Lembach (1953) is the most common model for evaluating central analgesic efficacy of drugs or compounds. The paws of mice are sensitive to heat at temperature which are not damaging to the skin, the response are usually jumping, licking of the paws and withdrawal (Mishra*, et al.*, 2011). The ability of Detarium microcarpum stem bark extract to prolong the reaction latency to thermally induced pain in mice by the hot plate further suggests central acting analgesic activity dose dependently (Khan *et al.*, 2010).The prolongation of the reaction time of the extract were comparable to that of standard drug used pentazocine which suggest that the extract may have a centrally acting property and mechanism by which it produced analgesia may be via central opoid receptors.

The anti-inflammatory activity of the extract was evaluated using carrageenan induced paw oedema in rats. Carrageenan induced hind paw oedema is the standard experimental model for acute inflammation. (Mishra*, et al.*, 2011) Carrageenan is the phlogistic agent of select for examining anti-inflammatory drugs as it is called to be antigenic and is devoid of afferent systemic effect, the model exhibits a high degree of

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reproducibility. This model is used in determining anti-inflammatory activities of medicinal agents and is well documented for various non-steroidal anti-inflammatory drugs (NSAIDs) (Sini *et al.*, 2010).

The extract at doses of 70 and 140 mg/kg caused significant inhibition of carrageenan-induced oedema in rats. Carrageenan induced inflammation is believed to be biphasic, the early phase (1-2 hours) is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings, the late phase is sustained by prostaglandins released and mediated by leukotrienes, polymorphonuclear cells, bradykinin and prostaglandins produced by macrophages tissue (Antonio and Brito, 1998). The inhibitory effect of the extract 140 mg/kg on carrageenan induced inflammation over period of 4 hours is similar to the effect of most non-steroidal anti-inflammatory drugs. This suggests that the extract acts in both early and later phase probably by inhibiting synthesis of arachidonic acid metabolites which produce oedema (Just *et al.*, 1998).

V. CONCLUSION

The methanol stem bark extract of Detarium microcarpum was found to possess several bioactive constituents associated with analgesic and anti-inflammatory activities. This partly justifies the claim for the traditional use of the plant in the management of pain and inflammatory conditions. The Detarium microcarpum methanol stem bark extract manifested with several toxicity when given orally for 28 days which include changes in biochemical and hematological parameters as well as changes in the morphology of liver, heart, kidney and spleen.

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