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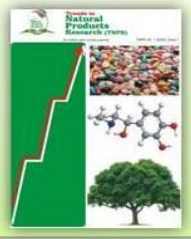

Evaluation of Anti-Inflammatory Activity of Methanol Leaf Extract of *Detarium senegalense* (J.F.Gmel) in Wistar rats

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Abstract: *Detarium senegalense* (J.F. Gmelin) commonly known as Tallow tree (Family, Fabaceae). In Nigeria, it is called *Taura* (Hausa), *Ogbogbo* (Yoruba), *Ofor* (Igbo). The study evaluated the anti-inflammatory activity of methanol extract of *Detarium senegalense* in rats. The phytochemical screening was conducted using standard protocols. Acute toxicity testing was carried out according to the OECD guidelines 420. Anti-inflammatory testing in rat was conducted by administering distilled water 1 ml/kg to group I rats, a standard drug (Piroxicam) 10 mg/kg to group II, and group III-V rats received 25, 50 and 100 mg/kg of methanol extract of *Detarium senegalese* (DS). Thirty minutes later an acute inflammation was induced by injecting 0.1ml (1 %) carrageenan into plantar surface of rat hind paw. The *Detarium senegalense* crude methanol extract (DSME) contains alkaloids, flavonoids, saponins, tannins and triterpenes. The Median Lethal Dose (LD_{50}) of DS was found to be above 2000mg/kg. In this test, DS 25, 50 and 100 mg/kg produced statistically significant ($p < 0.05$) decreased in the carrageanan-induced paw edema at 2 hours compared to D/W treated group. Also, the standard drug piroxicam 10mg/kg produced statistically significant ($p < 0.05$) decreased in the carrageanan-induced paw edema at 2 hours compared to D/W treated group. *Detarium senegalense* methanol extract possess anti-inflammatory activity and can be used for the development of herbal medicine for the treatment of inflammatory diseases. The crude extract should be fractionated and further tested using more anti-inflammatory models.

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INTRODUCTION

Inflammation is a local reaction which occur in active vascular tissues in response to internal or external stimuli. Therefore, inflammation is a protective process which occur in response to injury. In general, inflammation is not a disease but a symptom of a disease resulting from an inflammatory mediator's activity (Antonelli and Kushner, 2017; Chen *et al.* 2018; Bennett *et al.* 2018). Several diseases begin with inflammatory response at the initial stage and these include; syphilis, tuberculosis, leprosy, scleroma, cancer etc (Antonelli and Kushner, 2017; Chen *et al.* 2018; Bennett *et al.* 2018). There are a number of agents that trigger inflammation such as bacteria, viruses, change in temperature, injury, drugs, immunological disorders and metabolic disorders (Chen *et al.* 2018; Bennett *et al.* 2018). After cell injury, the brain usually responds to cell damage by directing the release of a number of local inflammatory mediators such as interleukin-1β (IL-1β), interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α), bradykinin, prostaglandins, leukotrienes, platelet-activating factor, which eventually cause inflammation (Antonelli and Kushner, 2017; Chen *et al.* 2018; Bennett *et al.* 2018). In the presence of inflammatory stimulus, phospholipase A_2 converts phospholipid to arachidonic acid which is subsequently converted to endoperoxides in the presence of cyclooxiginase enzymes. The endoperoxides finally produces prostaglandins which mediate pains and thromboxane A_2 which causes inflammation. (Chen *et al.* 2018; Bennett *et al.* 2018). Inflammation can be acute or chronic depending on the cause and duration. In acute inflammation there is presence of fluids, neutrophils, leukocytes, and plasma proteins predominantly at the site of injury (Antonelli and Kushner, 2017; Chen *et al.* 2018; Bennett *et al.* 2018; Yatoo *et al.* 2018; Furman *et al.* 2019). The major signs of acute inflammation include pain, swelling, redness, heat and loss of sensation (Antonelli and Kushner, 2017; Chen *et al.* 2018; Bennett *et al.* 2018). Chronic inflammation is a persistent inflammation that lasted for weeks. In this condition, tissue destruction and repair occur concurrently. The cellular component that contributed to chronic inflammation include monocytes, neutrophils, T-lymphocytes, and mast cells (Antonelli and Kushner, 2017; Chen *et al.* 2018; Furman *et al.* 2019).

Inflammation can be treated with both orthodox drugs and medicinal plants. Steroidal anti-inflammatory drugs (SAIDs) contain steroid hormones such as glucocorticoids (corticosteroids) which produces anti-inflammatory, immunosuppressive, and metabolic effects. Examples includes prednisolone, betamethasone and dexamethasone (Newman *et al.* 2017; Yatoo *et al.* 2018; Jahnavi *et al.* 2019). Non-steroidal anti-inflammatory drugs (NSAIDs) were discovered to have lesser side effects than SAIDs. They are classified mainly based on their mechanism of action which include nonselective cycloxyginase (COX) inhibitors, COX-I and COX-II selective inhibitors (Newman *et al.* 2017; Yatoo *et al.* 2018; Jahnavi *et al.* 2019). Nonselective COX inhibitors include aspirin, ibuprofen, naproxen, paracetamol, mefenamic acid, diclofenac, and piroxicam; COX-I selective inhibitors include ketorolac and indomethacin; COX-II selective inhibitors include celecoxib, rofecoxib, valdecoxib, etoricoxib, lumiracoxib and meloxicam (Newman *et al.* 2017; Yatoo *et al.* 2018; Jahnavi *et al.* 2019). Medicinal plant used traditionally in the management of inflammation include the following; *Azaridachta indica*, *Matricaria recutita*, *Phylanthus embellica*, *Berberis vulgaris*, *Ocimum basilicum*, *Curcuma longa*, *Moringa olifera*, *Crotalaria pallida*, *Ananas comosus* (Yatoo *et al.*2018).

The plant *Detarium senegalense* (JF Gmelin) commonly known as Tallow tree (Family Fabaceae). In Nigeria, it is called *Taura* (Hausa), *Ogbogbo* (Yoruba), *Ofor* (Igbo) (Sowemimo *et al.* 2011; Ukwubile *et al.* 2017; Sanni *et al.* 2018; Dossa *et al.* 2020). The bark of DS is used traditionally in the treatment of digestive problems, heavy bleeding, and pneumonia. The bark pulp is used in treating tuberculosis, wounds and skin infections. The leaves are used to treat constipation and fever (Sowemimo *et al.* 2011; Ukwubile *et al.* 2017). The fruit is used to treat leprosy, syphilis, cold, chest infections and it is also taken as food (Dossa *et al.* 2020). Experimental reports indicated that DS possess anthelminthic activity (Ukwubile *et al.* 2017). It also has anti-proliferative activity (Agada *et al.* 2018). Another experiment reported anti-microbial activity (Sowemimo *et al.* 2011). In addition, DS possess antidiarrheal activity (Sanni *et al.* 2018).

MATERIAL AND METHOD

Animals

Wistar Rats (160-180 g) of either sex were purchased from Department of Pharmacology and Therapeutics, Bayero University, Kano, Nigeria. They were kept under room temperature (25 ± 2 ºC) and 12-hours light/12-hours dark circle. The relative humidity was measured to be between 50 to 60 %. The animals were fed on Vital Feed (Bukuru, Jos) and water *ad libitum*.

Plant Materials

The whole plant material was collected from Ambana Town, Lafia Local Government, Nasarawa State. The identification and authentication were done by the Department of Plant Biology, Bayero

University, Kano. Voucher number was collected as BUKHAN 0343 and kept for future references.

Extraction

The leaves of DS were first washed, shade dried, and grinded into a coarse powder using mortar and pestle. The powdered fruit (1 kg) was macerated using 4L of 70% methanol v/v with occasional shaking for 7 days and filtered using Whatman No:10 filter paper. The filtrate was evaporated to dryness *in vacou* at 40ºC to yield residue (Deng *et al*. 2007).

Phytochemical Screening

The chemical composition of crude methanol extract was determined using phytochemical screening (Trease and Evans, 2002).

Acute Toxicity Study

This test was conducted according to the OECD guidelines 420 of 2001 (OECD, 2001). Five Wistar Rats weighing (160-180 g) were selected and divided into five groups of one rat each. Fixed doses of 5, 50, 300, 2000 and 5000 mg/kg of methanol of DSME extract was administered orally. The animals were observed for signs of toxicity and mortality within 48 hours. Further observation was made for up to two weeks for late signs of toxicity. The whole experiment was conducted between 900 hour and 1600 hour (OECD, 2001).

Carrageenan-Induced Paw Edema

Twenty-five Wistar rats were divided into 5 groups of 5 rats each. The group-I received distilled water 1 ml/kg, group-II the standard drug piroxicam 10mg/kg. Group-III to V received graded doses of DSME 25, 50 and 100mg/kg respectively. Thirty minutes later acute inflammation was induced by injecting 0.1ml (1%) carrageenan into plantar surface of rat hind paw. The paw volume was measured at 0, 1 ,2 ,3, 4 and 5 hours using a Vanier caliper to determine the diameter of edema (Winter *et al*. 1962).

Gas Chromatography/Mass Spectroscopy (GC/MS)

The analysis was conducted using GC machine (Hewlett Packard), a 6890-model series. The machine comprised of an ionization chamber, flame detector, and injector at a temperature of 250°C. The temperature was adjusted at 50°C for 5 minutes and later raised by 2°C/minutes gradually. The machine used helium gas 99.9%. The process begins with injection of 1 µm of the extract at a ratio of 1 to 30. The interpretation of the result was made using Library software database (Model 6890 series) was equipped with NIST14.L) (Thomas *et al*. 2013).

RESULTS

Phytochemical Constituents of Methanol Extract of *Detarium senegalense*.

The phytochemical constituents present in DSME are alkaloids, flavonoids, saponins, tannins and triterpenes (Table 1).

Median Lethal Dose (LD_{50})

The Median Lethal Dose (LD_{50}) of *Detarium senegalense* in rat using oral route was found to be above 2000 mg/kg.

Effect of *Detarium senegalense* on Carrageanan-Induced Paw Edema

In this test, DS 100 mg/kg produced statistically significant ($p < 0.05$) decreased in the carrageenan-induced paw edema at 2, 3 and 4 hours compared to D/W treated group. In addition, DS 50 mg/kg produced statistically significant ($p < 0.05$) decreased in the carrageenan-induced paw edema at 2 hours only compared to D/W treated group. Furthermore, DS 25 mg/kg produced statistically significant ($p < 0.05$) decreased in the carrageenan-induced paw edema at 1, 2 and 4 hours compared to D/W treated group. Also, the standard drug (piroxicam) 10mg/kg produced statistically significant ($p < 0.05$) decreased in the carrageenan-induced paw edema at 1, 2, and 4 hours compared to D/W treated group (Table 2).

Gas chromatography Mass Spectroscopy of *Detarium senergalense*.

The result of GC/MS reveals the presence of propane nitrile, 3-[4-Diethylamino-1-methyl-1-(1-methylethyl)-2-butynyloxy]- with molar of 250 g/mol, histamine, 5-nitro-N-trifluoroacetyl- with molar of 252 g/mol, histamine, N-acetyl-5-nitro- with molar of 198 g/mol, 8-methoxy-4-phenylquinoline-2-hydrazine with molar of 265 g/mol and N,N,N^1,N^1-Tetramethyl-3,6- acridine diamine with molar of 265 g/mol (Table 3).

Table1: Phytochemical Constituents of the Methanol Extract of *Detarium senegalense*.

Metabolite	Status
Alkaloid	+
Flavonoid	+
Saponin	+
Tannins	+
Steriods	-
Anthraquinone	-
Triterpenes	+

Key: + = Presence; - = Absence

Table 2: Effect of *Detarium senegalense* methanol extract on carrageanan-induced paw edema.

Treatment(mg/kg)	1hour	2hours	3hours	4hours	5hours
D/W 1	0.864±0.267	1.098±0.221	1.740±0.259	2.744±0.211	1.130±0.253
Piroxicam 10	2.1±0.267*	2.092±0.221*	1.740±0.259	1.456±0.211*	1.902±0.253
DS 100	1.990±0.267	2.856±0.221***	2.912±0.259*	1.776±0.211*	1.516±0.253
DS 50	1.788±0.267	2.508±0.221**	2.210±0.259	2.022±0.211	1.314±0.253
DS 25	2.508±0.267**	2.628±0.221***	2.790±0.29	1.456±0.211*	1.860±0.253

Data is presented as Mean ± S.E.M followed by Bonferoni Test for Multiple comparison at *p <0.05, **p <0.001, *** p < 0.0001. DW=(ml/kg), DS= *Detarium senegalense.*

Table 3: Gas chromatography Mass spectroscopy of *Detarium senergalense* Secondary Metabolites.

Chemical name	Organic formula	Molar Weight	Exact Mass	Database
Propanenitirile,3-[4-Diethylamino-1-methyl-1-(1-methylethyl)-2-butynyloxy]-	$C_5H_{26}N_20$	250	250.20	Spectrabase
Histamine, 5-nitro-N-trifluoroacetyl-	$C_7H_7F_3N_40_3$	252	252.05	NCBI
Histamine, N-acetyl-5-nitro-	$C_7H_{10}N_40_3$	198	198.08	PubMed
8-methoxy-4-phenylquinoline-2-hydrazine	$G_6H_{15}N_30$	265	265.12	PubChem
N,N,N^1,N^1-Tetramethyl-3,6- acridinediamine	$C_{17}H_{19}N_3$	265	265.16	PubChem

DISCUSSION

The secondary metabolites are responsible for the plant's pharmacological activity. During this study, the phytochemical constituents present in DSME were alkaloids, flavonoids, saponins, tannins and triterpenes. Similar result was obtained in other studies (Abdel Karim and Sakina, 2016; Ndiaye *et al.* 2016). The result of GC/MS using DSME reveals the presence of metabolites such as propanenitrile, 3-[4-Diethylamino-1-methyl-1-(1-methylethyl)-2-butynyloxy]-, histamine, 5-nitro-N-trifluoroacetyl-, histamine, N-acetyl-5-nitro- with, 8-methoxy-4-phenylquinoline-2-hydrazine and N,N,N^1,N^1-Tetramethyl-3,6- acridine diamine. These compounds were suggested to be responsible for the observed anti-inflammatory activity. Similar result was obtained in other studies (Abdel Karim and Sakina, 2016; Ndiaye *et al.* 2016).

In the course of this experiment, the median lethal dose (LD_{50}) of DS was estimated to be above 2000 mg/kg. This implies that the plant is moderately safe which justifies its consumption as food (Lorke, 1983). During the anti-inflammatory testing, carrageenan was use to induced inflammation in rats by triggering the release of inflammatory mediators such as interleukin, bradykinin, prostaglandins, and leukotrienes (Winter *et al*., 1962; Chen *et al.* 2018). Our study showed that DS 25, 50 and 100 mg/kg produced statistically significant decreased in the carrageenan-induced paw edema. The anti-inflammatory activity of DS was non-dose dependent but highest activity was observed at 25 mg/kg. The result was comparable to the outcome of other experiments (Adedapo *et al.* 2013; Hassan *et al.* 2013; Khuda *et al.* 2014; Okhuarobo *et al.* 2017; Mohammed *et al.* 2020). Notably, the standard drug piroxicam 10mg/kg produced statistically significant decreased in the carrageenan-induced paw edema at 1, 2, and 4 hours. However, the anti-inflammatory activity of DS at 25 mg/kg was superior to that of piroxicam. The outcome of this

study is related to the result of another study (Ayanwuyi *et al.* 2010).

RECOMMENDATIONS

Plant extract should be fractionated, sub chronic toxicity should be conducted, and more anti-inflammatory models carried out.

CONCLUSION

Detarium senegalense methanol extract possess anti-inflammatory activity and can be used for the development of herbal medicine for the treatment of inflammatory diseases.

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