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In vivo Anti - inflammatory and toxicity studies on crude leaf extracts of Dalbergia hancie Benth

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Abstract: Traditional healers in the eastern part of Nigeria use Dalbergia hancie to treat majorly inflammation. The efficacy of this plant for this use has not been experimentally proven. Three solvents of different polarities; n- hexane, ethylacetate and methanol were used to extract biomolecules by soxhlet extraction and phytochemical analysis were carried out on the crude extracts. The anti - inflammatory activity was carried out using the method described by Paulrayer et al (2019) with little modification. The acute toxicity was conducted using the method described by Enegide et al (2013). The phytochemical tests showed the presence of steroids, flavonoids tannins and saponin in hexane, ethyl acetate and methanol crude extracts. The results of anti-inflammatory activity showed that n- hexane extract gave a decreasing percentage inhibition of inflammation 30 % at 1 hr, 18.8 % at 2 hrs, 13.3 % at 3 hrs, 14.29 % at 4 hrs, 14.29 % at 5 hrs, ethylacetate produces increasing percentage inhibition as follows; 14.3 % at 1 hr, 31.3 % at 2 hrs, 33.33 % at 3 hrs, 42.86 % at 4 hrs, 50 % at 5 hrs and methanol extract possess increasing percentage inhibition properties over inflammation; 24.3 % at 1 hr, 40 % at 2 hrs, 36 % at 3 hrs, 45.7 % at 4 hrs and 60 % at 5 hrs. In the toxicity test, extracts from the leaf of Dalbergie hancie were administered to mice at different concentrations of 10 mg/kg, 100 mg/kg, 1000mg/kg, 2000 mg/kg, 3000 mg/kg, 4000 mg/kg and 5000 mg/kg. There were no sign of toxicity and no death was recorded on the mice. These results authenticated the use of Dalbergia hancie as a remedy for various inflammatory disorder by the traditional healers, and that it is safe to take extracts from the leaf of Dalbergia hancie .

Key words: Dalbergia hancie, phytochemical, anti - inflammation, toxicity

Introduction

Dalbergia hancie, locally called Mgbehuhu by some parts of South Eastern Nigeria, is a hedge shrub mostly used in fencing of barns and yards for the demarcation of boundaries by the people of Enugu State, South Eastern Nigeria. They are also used as rafters and poles in construction sites, walking sticks for the elderly and the wounded, drumsticks, arrowheads, pestles, traditional cups and plates owing to their high tensile strengths. (Lemmens, 2008).

Dalbergia hancie belongs to the family of Legumes which include a large number of domesticated species harvested as crops for human and animal consumption as well as for oils, fibre, fuel, fertilizers, timber, medicines, chemicals, and horticultural varieties (Lewis et al., 2005).

Most Dalbergia species have been shown to contain phytochemicals that are potent in the treatment of different ailments. These include tannins, flavonoids, saponins, reducing sugars, terpenoids, glycosides, and proteins, (Faiza et al.; 2019). Dalbergia ecastaphyllum contains phenol, and flavonoids, and has been confirmed to possess antioxidant activity. The resin of D. ecastaphyllum contains isoflavonoids and other phenolics (Jairo et al.; 2020). Dalbergia sissoo have vast medicinal properties like abortifacient, aphrodisiac, anthelmintic, antipyretic agents. It can also be used as anti-vomiting agent, in the treatment of ulcers, dysentery, stomach problems and skin diseases. (Kirtikar and Basu, 1993). In rural areas, D. sissoo are also used as folk medicine in various condition like excoriations and gonorrhea. Anti-pyretic and analgesic activities of leaf of D. sissoo have also been reported (Hajare et al.; 2000). In ayurveda, the leaf juice is also used in eye treatment. There are

Significant anti-inflammatory effects without any side effect which have been reported on the alcoholic leaf extracts of D. sissoo (Hajare et al.; 2001). Animal non- specific diarrhoea can also be treated by using leaves of D.sissoo. In humans sore throats, heart problems, dysentery, syphilis and gonorrhea were also treated using the aqueous leaf extract. Anti-oxidant activity better than regularly used anti-oxidants like Selenium and vitamin E have also been reported. A treatment of colorectal cancer using the leaf extract has better significant effect than the conventional treatment(Hajareet al.; 2001). In some areas, D. sissoo and Dhatura stramoniumare combined with cow urine are used for the prevention and the treatment of chronic bacterial infection. (Hajare et al.; 2001)
In all these reports, little or no studies have been conducted on D .hancie although the plant has have been used for ages by traditional healers in reducing inflammation due to trauma and other disorders. The present study seeks to authenticate this claim of its use as herbal remedy for anti-inflammatory purposes.

Materials and methods

Collection of Materials

The fresh leaves of Dalbergia hancie were collected from a herbalist, authenticated by the Botany Department of Nnamdi Azikiwe University Awka and deposited in the Herbarium of the Department with herbarium number 201A. The leaves were washed and dried under shade to prevent the loss or destruction of active components. The dried leaves were pulverised and stored in a glass container.
Wistar rats (200–300 g) procured from an animal breeder in Awka Anambra State, were kept in the Animal House at the Faculty of Pharmacy, Department of Pharmacology, Agulu, Anambra State, Nigeria for acclimatization.

Extraction of the plant material

The extraction of the leaves (200 g) of Dalbergia hancie Benth was carried out using n- hexane, ethyl acetate and methanol (600 cm^3 each) with a Sohxlet extractor.

Phytochemical Screening

The extracts were subjected to phytochemical identification of different compounds (Sowore, 1993, Akpuaka, 1999, Trease and Evans, 2002). The presence of alkaloids was tested using, Dragendorf's test. The presence of steroids was investigated by Liebermann Buchard test. Test for flavonoid was carried out using 10 % sodium hydroxide. Frothing test was used to test for saponins. Test for tannins was carried out using ferric chloride. Other phytochemicals that were tested are glycoside, anthracene and resin.

ACUTE INFLAMMATION

The systemic acute anti-inflammation activity of crude leaf extracts of Dalbergia hancie Benth was studied using the method described by Winter et al 1962.The rats were grouped into five per group. Group 1 (the control) received 10 ml/kg of distilled water, other groups received 100 mg/kg of the extracts. After One hour post - treatment, the animals received sub - plantar injection of 0.1 ml of fresh egg albumin in the right hind paw. The paw size was measured before (basal) and at 1, 2, 3, 4 and 5 hours after egg albumin injection using fluid displacement method. Increase in the paw volume was quantified as inflammation. The percentage inhibition was calculated using the formula

$100\ (1 - \frac{a-x}{b-y})$

a = mean paw volume of treated rats at various time intervals after egg albumin injection
x = mean paw volume of treated rats before egg albumin injection
b = mean paw volume of control rats at various time interval after egg albumin injection
y = mean paw volume of control rats before egg albumin injection

THE ACUTE TOXICITY (LD_{50})

Acute toxicity, LD_{50} was carried out according to the method of Enegide et al 2013. In this method, a total of 13 mice were and the analysis was carried out in two phases. Phase 1: Nine mice were used and they were grouped into three mice each. Group 1 received 10 mg/kg of the extracts orally. Group two received 100 mg/kg , while group three received 1000 mg/kg. The animals were constantly monitored for one hour, intermittently for the next three hours and after twenty - four hours for behavioural changes and mortality. From the results of the first phase, the second phase was carried out. In this phase, four mice were used, and were treated separately. The first mouse received 2000 mg/kg, second received 3000 mg/kg, third received 4000 mg/kg

while the fourth received 5000 mg/kg. The animals were monitored as in phase one for behavioural changes and mortality.

Results and Discussion

Table 1: yield of extracts from the solvents

Extracting solvents	Weight of extracts	Yield %
Hexane	4.67	2.34
Ethyl acetate	11.66	5.83
Methanol	15. 20	7.60

Table 1: Weight and percentage yield of crude extracts from the three solvents.

After the extraction and drying of the extracts, the methanol extract had the highest yield 15.20g (7.60 %), followed by ethylacetate extract (11.66 g and 5.83 %) and hexane (4. 67 g 2. 34 %). From the result, the most polar solvent extracted more components than the less polar solvent, methanol was the best extracting solvent than the other two, while ethylacetate was a better extracting solvent than n - hexane.

Phytochemical analysis of the crude extracts of Dalbergia hancie

Table 2 : phytochemical results for crude extract of Dalbergia hancie Benth

	Hexane	Ethylacetate	Methanol
Saponins	+	+	+
Glycosides	-	-	-
Tannins	+	+	+
Flavonoids:	+	+	+
Anthraquinones	-	-	-
Steroids	+	+	+
Alkaloids:	-	+	+
Resin	-	-	-

+ = Present, - = Absent

Table 2 gives the result of the phytochemical studies conducted on the crude extract of D. hancie Benth. The phytochemical analysis of n- hexane crude extract showed that saponin, tannin, flavonoid, and steroids were present. In the ethyl-acetate crude extract, saponin, tannins, flavonoid, steroid and alkaloid were present, and methanol crude extract showed the presence of saponin, tannins, flavonoids, steroids and alkaloids.

Flavonoids are pigments that give most flowers, fruits, and seeds colour. Flavonoids are commonly dispersed in plants with different metabolic functions (Heller and Forkmann,1999). Flavonoids are known for their anti - inflammation, antimicrobial, antioxidant, antiviral and antifungal activities. These flavonoids include Quercetin, morin, rutin, dihydroquercetin (taxifolin), apigenin, catechin and hesperidin are known to be potent in fighting ailment (Selway, 1986). Dalbergia species are known to contain flavonoids as seen in Dalbergia hancie. Some Dalbergia Species like D. sissoo contains flavonoid and experimental tests has shown that D. sissoo possesses anti- inflammatory, antimicrobial, antioxidant and anti-diarrhoeal activities (Hajare et al.; 2001). Dalbergia ecastaphyllum also contains isoflavonoids and benzophenone, these derivatives of flavonoids are key to the potency of Dalbergia species in traditional medicine (Jairo et al.; 2020).

Dalbergia hancie contain tannins which are great nutraceuticals in order to avert different diseases such as cancer, cardiovascular, kidney diseases, and diabetes. Major sources of tannins are fruits, vegetables, bark, wood, leaves, and seeds such as green tea, apples, cocoa, chocolate, grapes, apricots, and cherries. The green tea contains condensed tannin namely epigallocatechin gallate (EGCG) and epicatechin gallate (ECG), which have shown anticancer activity in terms of inflammatory and antioxidant properties both in vivo and in vitro experimental systems (Smeriglio et al.; 2017). The Dalbergia species have been shown to be non-toxic to Wister rat at a very high concentration as discovered in D. sissoo when the extract is administered at 3000 mg/kg concentration(Mohammed and Arun, 2016).

Alkaloids such as nicotine and anabasine are used as insecticides. Several alkaloids act on the nervous system. Plants that contain protoberberine alkaloids are used as analgesics, antiseptics, sedatives, and stomatic in Chinese folk medicine. In Indian and Islamic folk medicine, such plants are used for bleeding disorders and eye diseases, and antiseptics, sedatives, stomatics, and uterine muscle depressants (Seigler, 1998). Some work carried on D. sissoo has shown that it possesses analgesic and antipyretic activities (Ali, 2017). The Dalbergia hancie which has given a positive test for alkaloid could be used as analgesics, antiseptics, sedatives, and stomatics depending on the method of preparation of the drug.

Dalbergia species contain saponins and sapogenins which possess virucidal activity and are capable of deactivating viruses, for example, D. sissoo has antiviral activity, purified saponin mixture from Maesa

lanceolata (Morrissey and Osbourn, 1999, Sindambiwe et al.; 1998. Mengoni et al.; 2002). Some saponins have anti - inflammatory activity, this is because of the inhibition of the mediators of inflammation such as histamine, serotonin and prostaglandin along with its antioxidant property which inhibits the formation of ROS which also plays a main role in inflammation (Sayyah et al.; 2004, Tatiya et al.; 2007).

There are many effect of corticosteroids which include alterations in carbohydrate, protein lipid metabolism; maintenance of fluid and electrolyte balance; and protection of normal function of the cardiovascular system, the immune system, the kidney, skeletal muscle, the endocrine system, and the nervous system. Also, corticosteroids provide the organism with the ability to resist such stressful circumstances as noxious stimuli and environmental changes (Bruton et al.; 2006). The Dalbergia family are known to contain essential phytochemicals which traditional healers utilise in the treatment of diseases. Dalbergia hancie contain steroids, the presence of the phytochemicals in plant have been shown to be potent in treating diseases. The phytochemical constituent of D. hancie is similar to the family like D. sissoo which showed the presence of Alkaloids, Carbohydrates, saponins, flavonoids, glycosides and steroids (Sagar and Upadhyaya, 2010). Dalbergia ecastaphyllum has been shown to contain fatty acid, total phenol and flavonoid (Jairo et al., 2020). These phytochemical in Dalbergia species show aggression against organisms causing diseases.

The inflammation inhibition of hexane, ethyl acetate, methanolic extracts of Dalbergia hancie

Table 3: The inflammation inhibition result of the hexane, ehtylacetate, methanol extracts of Dalbergia hancie

Sample	Basal	1 hour	2 hours	3 hours	4 hours	5 hours
CH	0.50±0.00	1.20±0.05	1.15±0.06	1.15±0.03	1.10±0.02	1.10±0.06
CE	0.50±0.00	1.10±0.02	1.05±0.06	1.0±0.08	0.9±0.04	0.85±0.06
CM	0.57±0.02	1.10±0.06	1.05±0.03	1.05±0.02	0.95±0.02	0.85±0.06
Diclofenac	0.60±0.00	1.00±0.02	0.90±0.06	0.80±0.02	0.65±0.02	0.65±0.02
Distilled water10 ml/kg	0.50±0.00	1.20±0.02	1.30±0.03	1.25±0.04	1.20±0.02	1.20±0.02

CH = crude n- hexane extract, CE = crude ethylacetate extract, CM = crude methanol extract

Values expressed as MEAN ± S.E.M, n = 5 in each group, *P <0.05 compared with control.

The anti- inflammatory activity of the leave extracts of Dalbergia hancie was moderate as compared to the inhibitory action of diclofenac. There were increase in all the basal paw from 0 hour to one hour in the animals. There was noticeable decrease in the basal after 2 hours of treatment in all the extracts and the standard used. The extracts anti - inflammatory activity continued to increase from 2 hour to 5 hours.

Diclofenac inhibits the thromboxane - prostanoid receptor, affect arachidonic acid release and uptake, inhibit lipoxygenase enzymes, and activate the nitric oxide - cGMP antinociceptive pathway. It can also inhibit the substrate P, and peroxisome proliferator activated receptor gamma (PPAR gamma), blockage of acid - sensing ion channels, alteration of interleukin - 6 production, and inhibition of N - methyl - D - aspartate (NMDA) receptor hyperalgesia (Gan, 2010). Therefore, it is likely that the leaf extract of D. hancie might suppress the preparation of these substances or inhibit the action of these substances and thus exert the anti- inflammatory activity.

Table 4: The percentage inflammation inhibition result of hexane, ethyl acetate methanolic extracts of Dalbergia hancie

Sample 100 mg/kg	1 hour	2 hours	3 hours	4 hours	5 hours
CH	30.00	18.80	13.30	14.29	14.29
CE	14.30	31.30	33.33	42.86	50.00
CM	24.30	40.00	36.00	45.70	60.00
Diclofenac	42.90	62.50	73.33	92.86	92.86

CH = crude n- hexane extract, CE = crude ethylacetate extract, CM = crude methanol extract

Values expressed as MEAN ± S.E.M, n = 5 in each group, *P <0.05 compared with control

The results in table 4 have shown the percentage inhibition of inflammation ability of extracts of three solvents of different polarities. The non - polar solvent n- hexane was most effective in its anti - inflammatory activity at the first hour, and loses its potency as time increases. Ethylacetate which is moderately polar showed an increase inhibition against inflammation and has its highest anti - inflammation at 5 hours. Methanol extract possessed increased anti-inflammatory activity. It showed the most inhibitor effect to inflammation at 5 hours compared to other extracts from Dalbergia hancie.

The solvents used most time by herbalists are water and alcohols to extracts biomolecules. This result has shown that the use of Dalbergia hancie to treat inflammation could be correct and that as the polarity of the

solvent is increasing, polar biomolecules like phenolics are being extracted which possesses anti - inflammatory activity. The use of non-steroidal anti-inflammatory drug (NSAID) have been reported to be the medication used by clinicians for joint and spine related inflammatory pain. Increased concentrations of tumor necrosis TNF-α are said to be the reason of the cardinal signs of inflammation to occur. NSAID was said to have the mechanisms that are primarily through interaction with proinflammatory cytokines interleukin (IL) and tumour necrosis factor (TNF-α) (Ghosh et al.; 1998). The extracts from D. hancie could be potential inhibitors of the agents causing inflammation and break the pathways for the agglomeration of these substances.

Table 5: Acute toxicity (LD_{50}) of haxane, ethyl acetate and methanol extract of Dalbergia hancie Benth

Phase	Dose	Number of deaths
1	10 mg/kg	0/3
	100 mg/kg	0/3
	1000 mg/kg	0/3
2	2000 mg/kg	0/1
	3000 mg/kg	0/1
	4000 mg/kg	0/1
	5000 mg/kg	0/1

Table 5 gives the result of acute toxicity of extracts from Dalbergia hancie. The first group of the mice received 10 mg/kg, 100 mg/kg and 1000 mg/kg extracts of n- hexane, ethylacetate and methanol orally. The mice were observed after the first 4 hours and there was no sign of toxicity. They were then observed subsequently for 24 hours, and no sign of toxicity was noticed. The second group were administered with higher dosage of 2000 mg/kg, 3000 mg/kg, 4000mg/kg and 5000 mg/kg. The toxicity was monitored as in the first stage, and there was no sign of toxicity or death recorded. The oral LD_{50} mice was found to be greater than 5000 mg/kg.

The Dalbergia species are widely used in traditional medicine and had been found to be safe drugs. Dalbergia sissoo was investigated for acute toxicity study, the result revealed that ethanolic extract was not toxic up to 3000 mg/kg body weight (Mohammed and Arun, 2016). Dalbergia saxatilis extract was administered to Wistar rats and mice at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg. The median lethal dose in both mice and rats was found to be above 5000 mg/kg (Hassan et al.; 2015).

Conclusion: The phytochemicals present in Dalbergia hancie include, saponins, glycosides, alkaloids, flavonoids, tannins, and steroids. The treatment for anti-inflammatory activity has shown that the methanol extract is most effective, hence, accounting for the use of polar solvents such as water and alcohols by traditional healers to prepare the extracts for use. The toxicity test has shown that leaves of Dalbergia hancie non toxic.

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