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Investigation of Anti-inflammatory Activity of *Guizotia abyssinica* (L.f.) Cass. leaves and seed

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Abstract

Guizotia abyssinica (L.f.) Cass. Syn. *G. oleifera* D.C., *Polymnia abyssinica* L.f., Suppl., *Verbesina sativa* Roxb., *Jaegeria abyssinica* Spr., commony known as Ramtil (H) and Niger (E) belongs to family Asteraceae had been widely used for its reported biological activities in indigenous system of medicine. The present investigation was carried out to find the effect of aqueous and ethanolic extract of leaves and seed of *Guizotia abyssinica* for its anti-inflammatory activity in rodents. The anti-inflammatory activity was evaluated using acute inflammatory models viz., carrageenan induced paw oedema. Oral administration of the extract at the doses 100 and 200 mg/kg b.w. exhibited dose dependent and significant anti-inflammatory activity in ($p < 0.01$). Hence, present investigation established pharmacological evidences to support the folklore claim that *Guizotia abyssinica* is used as anti-inflammatory agent.

Key-Words: *Guizotia abyssinica*, Leaves, Seed, Anti-inflammatory activity

Introduction

Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation[1]. Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow[2]. Several experimental models of paw oedema have been described. Carrageenan-induced paw oedema is widely used for determining the acute phase of inflammation.

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Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation[3] whereas prostaglandins are detectable in the late phase of inflammation[4]. Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs possess well known side and toxic effects. Moreover, synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. Exploring the healing power of plants is an ancient concept. For centuries people have been trying to alleviate and treat disease with different plant extracts and formulations[5]. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs[6]. Screening of the plants for their biological activity is done on the basis

of either their chemotaxonomic investigation or ethnobotanical knowledge for a particular disease. Identification of a particular compound against a specific disease is a challenging long process.

Guizotia abyssinica (L.f.) Cass. Syn. *G. oleifera* D.C., *Polymnia abyssinica* L.f., Suppl., *Verbesina sativa* Roxb., *Jaegeria abyssinica* Spr., commony known as Ramtil (H) and Niger (E) belongs to family Asteraceae. It is native of Abyssinica (South Africa). It is cultivated widely as an oil seed crop in India, Ethiopia, Abyssinica and parts of East Africa. In India, it is grown extensively in Madhya Pradesh, Hyderabad, Orissa, Bombay and Mysore and to some extent in Bihar, Madras and Vindhya Pradesh. It is an erect, stout, branched annual herb, grown for its edible oil and seed. Its cultivation originated in the Ethiopian highlands, and has spread to other parts of World. The seed, technically a fruit called an achene, is often sold as bird feed. The leaves are arranged on opposite sides of the stem. At the top of the stem leaves are arranged in an alternate fashion. Leaves are 10-20 cm long and 3-5 cm wide. The leaf margin morphology varies from pointed to smooth and leaf colour varies from light green to dark green, the leaf surface is smooth. Flower is yellow and, rarely, slightly green. The heads are 1.5-5 cm in diameter with 0.5-2 cm long ray florets. Two to three flower-heads grow together, each having ray and disk florets. The receptacle has a semi-spherical shape and is 1-2 cm in diameter and 0.5-0.8 cm high. The receptacle is surrounded by two rows of involucral bracts. The head consists of six to eight "petals" (fertile female ray florets). The disk florets, usually 40-60 per flower-head, are arranged in three whorls. The disk florets are yellow to orange with yellow anthers, and a densely hairy stigma.[7]

The plant is used by the various tribal communities of India in the treatment of various disease and disorders, keeping this view the present work was conceived to explore the folk lore and traditional uses of this plant. As there is no reference in literature to the anti-inflammatory aspects, it was considered worthwhile to study the anti-inflammatory activity of aqueous and ethanolic extract leaves and seed of *Guizotia abyssinica* in rodents.

Material and Methods

Selection, collection and authentication of plant/plant material

The seeds of the selected plant were collected in the months of July 2011 from the Jawahar Lal Nehru Krishi Vishwavidhalay (JNKVV) Agriculture University, Jabalpur, M.P. and identified & authenticated by Dr. (Mrs.) Neeta Singh, Prof. and Head, Department of Botany, Govt. Girls PG College, A.P.S. University, Rewa, M.P. and was deposited in our Laboratory, Voucher specimen No. PCog/GA/0914. The seeds were then sown in soil, irrigated regularly and after 3-4 months leaves was collected, dried under shade, powdered and stored in an air-tight container for further use.

Preparation of Extract

Extraction of Leaves

Sample were shattered and screened with 40 mesh. The shade dried coarsely powdered leaves of *Guizotia abyssinica* (L.f.) Cass. (250gms) was loaded in Soxhlet apparatus and was extracted with petroleum ether (60-62°C), Chloroform, ethanol and water until the extraction was completed. After completion of extraction, the solvent was removed by distillation. The extracts were dried using rotator evaporator. The residue was then stored in dessicator and percentage yield were determined.[8]

Extraction of Seed

Sample were shattered and screened with 40 mesh. The shade dried coarsely powdered seeds of *Guizotia abyssinica* (L.f.) Cass. (250gms) was loaded in Soxhlet apparatus and was extracted with petroleum ether (60-62°C), Chloroform, ethanol and water until the extraction was completed. After completion of extraction, the solvent was removed by distillation. The extracts were dried using rotator evaporator. The residue was then stored in dessicator and percentage yield were determined.[8]

Acute Toxicity Studies of Extracts

The mice were used for acute toxicity study as per OECD guidelines 423. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee after scrutinization.[9]

Anti-inflammatory Activity

Carrageenan induced paw oedema

Animals

Adult albino rats of both sex (200-250 gm) were procured from Veterinary College, Mhow, Indore, (M.P.) maintained under ideal feeding and management practices in the laboratory. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light.

The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee after scrutinization.

Study Design

The animals were divided into 10 groups each containing six animals. Group I served as untreated control and received 0.9 normal saline, group II served as positive control and received Indomethacin (10 mg/kg, i.p.) and others group were treated with different doses of *G. abyssinica* aqueous and ethanolic extracts.[10]

Anti-inflammatory Screening

The aqueous and ethanolic extract of *Guizotia abyssinica* and standard drug Indomethacin were administered in prescribed doses. Control received 0.1 ml of 1% carrageenan in normal saline. The administration of extract and drug was 30 min prior to injection of 0.1 ml of 1% carrageenan in the right hind paw subplatar of each rat. The paw volume was measured plesthysmometrically (model 7140, Ugo Basil, Italy). Prior to injection of carrageenan, the average volume of the right hind paw of each rat was calculated. At 1, 2, 3, 4, 5 and 6 hr after injection paw volume was measure. Reduction in the paw volume compared to the vehicle-treated control animals was considered as anti-inflammatory response.[10-11]

Statistical analysis

All the values ware statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test. Comparison between control and drug treated groups were considered to be significant (*P<0.01). All values are expressed as mean ± SEM.

Results and Discussion

The aqueous and ethanolic extracts of leaves and seeds of plant of *Guizotia abyssinica* (L.f.) Cass. were screened for acute toxicity study by OECD guideline no. 423 for determination of LD_{50}. The results showed that the aqueous and ethanolic extracts i.e., AEGAL, EEGAL, AEGASe and EEGASe were belonging to category-5(unclassified). Hence, LD_{50} was 5000 mg/kg, therefore, ED_{50} was 500 mg/kg. Therefore, two doses of 100 and 200 mg/kg was considered for present investigation.

The aqueous and ethanolic extract of *Guizotia abyssinica* leaves and seed were evaluated for anti-inflammatory activity in animal models and the results are summarized in Table 1 and 2. The result obtained indicates that the extract found to have significant ($P < 0.01$) anti inflammatory activity in rats. The AEGAL and EEGAL at the test doses 100 and 200 mg/kg b.w. reduced the oedema induced by carrageenan by 42.68%, 47.56%, 35.97% and 39.63% respectively at 6 h, whereas the AEGASe and EEGASe at the test doses 100 and 200 mg/kg b.w. reduced the oedema induced by carrageenan by 47.56%, 53.04%, 37.19% and 45.73% as compared to standard drug which showed 63.41% of inhibition as compared to the control group.

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, whereas plants still hold their own unique place, by the way of having no side effects. Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation so as to exploit them as herbal anti-inflammatory agents. The enzyme, phospholipase A2, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting polymerphonuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase A2 converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cyclooxygenase (prostaglandin synthesis) to prostaglandins, which are major components that induce pain and inflammation[12]. It is well known that carrageenan induced paw edema is characterized by biphasic event with involvement of different inflammatory mediators. In the first phase (during the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin play role, while in

second phase (3 – 4 h after carrageenan injection). Kinin and prostaglandins are involved[13]. From the above studies it is quite apparent that the aqueous extract possesses significant anti-inflammatory activity. The study justifies its use in inflammation as suggested in the folklore medicines

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Table 1: Effect of *Guizotia abyssinica* (L.f.) Cass. extracts on carrageenan induced oedema

Treatment	Dose (mg/kg)	Right hind paw volume (mL)					
		1 h	2h	3h	4h	5h	6h
C	-	1.70±.01	1.82±0.00	1.72±0.00	1.66±0.01	1.66±0.00	1.64±0.00
SD	10	1.20±0.01	0.83±0.01	0.77±0.02	0.73±0.01	0.73±0.01	0.60±0.01
AEGAL	100	1.45±0.03	1.22±0.01	1.22±0.01	1.16±0.00	1.04±0.00	0.94±0.01
AEGAL	200	1.40±0.00	1.18±0.00	1.18±0.01	1.10±0.01	0.89±0.00	0.86±0.04
EEGAL	100	1.61±0.02	1.57±0.00	1.41±0.01	1.39±0.01	1.21±0.00	1.05±0.01
EEGAL	200	1.57±0.01	1.55±0.01	1.40±0.01	1.30±0.00	1.19±0.00	0.99±0.01
AEGASe	100	1.31±0.02	1.22±0.01	1.13±0.02	1.06±0.01	1.00±0.01	0.86±0.02
AEGASe	200	1.23±0.02	1.20±0.01	1.03±0.04	1.02±0.01	0.91±0.91	0.77±0.00
EEGASe	100	1.53±0.05	1.50±0.01	1.39±0.00	1.21±0.01	1.05±0.02	1.03±0.03
EEGASe	200	1.45±0.01	1.36±0.01	1.29±0.00	1.10±0.01	0.99±0.00	0.89±0.02

Values are expressed as X (Mean) ±SEM, n=6. (One way ANOVA followed by Dunnett Multiple Comparison Test). Statistically significance *P<0.01 in comparison to control.

Abbr.: C=Control, SD=Standard drug (Indomethacine), AEGAL = Aqueous extract of *Guizotia abyssinica* Leaves, EEGAL= Ethanolic extract of *Guizotia abyssinica* Leaves, AEGASe = Aqueous extract of *Guizotia abyssinica* Seed, EEGASe = Ethanolic extract of *Guizotia abyssinica* Seed

Table 2: Percentage inhibition of *Guizotia abyssinica* (L.f.) Cass. extracts on carrageenan induced oedema

Treatment	Dose (mg/kg)	Percentage inhibition at different interval					
		1 h	2h	3h	4h	5h	6h
C	0	-	-	-	-	-	-
SD	10	54.39	55.23	55.23	56.02	56.02	63.41
AEGAL	100	14.70	32.96	29.06	30.12	37.34	42.68
AEGAL	200	17.64	35.16	31.39	33.73	46.38	47.56
EEGAL	100	5.29	13.73	18.02	16.26	27.10	35.97
EEGAL	200	7.64	14.83	18.60	21.68	28.31	39.63
AEGASe	100	22.94	32.96	34.30	36.14	39.75	47.56
AEGASe	200	27.64	34.06	40.11	38.55	45.18	53.04
EEGASe	100	10.00	17.58	19.18	27.10	36.74	37.19
EEGASe	200	14.70	25.27	25.00	33.73	40.36	45.73

Abbr.: C=Control, SD=Standard drug (Indomethacine), AEGAL = Aqueous extract of *Guizotia abyssinica* Leaves, EEGAL= Ethanolic extract of *Guizotia abyssinica* Leaves, AEGASe = Aqueous extract of *Guizotia abyssinica* Seed, EEGASe = Ethanolic extract of *Guizotia abyssinica* Seed

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