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Original Research Article

Evaluation of Analgesic Activity by Acetic Acid Induced Writhing Method of Crude Extracts of *Acacia nilotica*

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Abstract: Pain may be defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage. It may vary in intensity (mild, moderate, or severe), quality (sharp, burning or dull), duration (transient, intermittent, or persistent) and referral (superficial or deep, localized or diffuse).Typically, it is a direct response to an untoward event associated with tissue damage, such as injury, inflammation or cancer, but severe pain can arise independently of any obvious predisposing cause (e.g. trigeminal neuralgia) or can persist long after the precipitating injury has healed (e.g. phantom limb pain). In addition some disorders commonly occur in patients who experience pain such as – hyperalgesia (extreme sensitivity to pain stimuli), allodynia (pain in response to non-noxious mechanical stimuli) and hyperesthesia (abnormal sensitivity to sensory stimuli). In recent times, medicinal plants occupy a considerable position for being the paramount sources of drug discovery irrespective of its categorized groups- herb, shrub or tree**.** The crude methanolic extract of *Acacia nilotica* bark with different soluble partitionates were subjected to investigate for the evaluation of analgesic, hypoglycemic, CNS depressant and antidiarrheal activity on mice and thrombolytic, antihelmentic, antimicrobial, antioxidant along with cytotoxicity different in vivo experiment. Analgesic activity of the plant *Acacia nilotica* was evaluated by acetic acid Induced writhing method. The result showed that the bark extract of plant *Acacia nilotica* possessed moderate analgesic activity.
Keywords: extreme sensitivity, analgesic, hypoglycemic, CNS depressant.

INTRODUCTION

Pain may be defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. It may vary in intensity (mild, moderate, or severe), quality (sharp, burning or dull), duration (transient, intermittent, or persistent), and referral (superficial or deep, localized or diffuse).Typically, it is a direct response to an untoward event associated with tissue damage, such as injury, inflammation or cancer, but severe pain can arise independently of any obvious predisposing cause (e.g. trigeminal neuralgia) or can persist long after the precipitating injury has healed (e.g. phantom limb pain) [1]. In addition some disorders commonly occur in patients who experience pain such as – hyperalgesia (extreme sensitivity to pain stimuli), allodynia (pain in response to non-noxious mechanical stimuli) and hyperesthesia (abnormal sensitivity to sensory stimuli). A number of compounds with analgesic activity have been isolated so far from different plant origin which led the scientists to uncover this therapeutic area with newer molecule with better pharmacokinetic and Pharmacodynamics profile. As *Acacia nilotica* has been used in folk medicine as a cough healer, anti-diarrheal agent the aim of this study was to investigate analgesic effects of its methanolic (MeOH) extract of bark and its different fractions.

THE PLANT FAMILY: FABACEAE [3] (a, b)

The plant under investigation is *Acacia nilotica* belongs to the family Fabaceae. The **Fabaceae**, also called Leguminosae or bean and pea family, is the third largest family in terms of agricultural and economic importance. Legumes includes a large number of domesticated species harvested as crops for human and animal consumption as well as for oils, fiber, fuel, fertilizers, timber, medicinals, chemicals, and horticultural varieties[4]. In addition, the family includes several species studied as genetic and genomic model systems.

GROWTH PATTERN

Legumes vary in habit from annual and perennial herbs to shrubs, trees, vines/lianas, and even a

few aquatics. Ranging in size from some of the smallest plants of deserts and arctic/alpine regions to the tallest of rain forest trees, legumes are a conspicuous, and often dominant, component of most of the vegetation types distributed throughout temperate and tropical regions of the world [5]. Legumes are particularly diverse in tropical forests and temperate shrub lands with a seasonally dry or arid climate. This preference for semi-arid to arid habitats is related to a nitrogen demanding metabolism. While many species have the ability to colonize barren and marginal lands because of their capacity to "fix" atmospheric nitrogen via a symbiotic association with root-modulating bacteria, this is just one of several ways in which legumes obtain high levels of nitrogen to meet the demands of their metabolism [6]. Over the past 30 years, the study of legume classification and biology has benefitted from major advances in understanding of the morphology, evolution and systematics, and ecology of the family [7].

CHARACTERISTICS

Morphologically, Fabaceae is characterized by leaves simple to compound (pinnate, rarely palmate, or bipinnate), unifoliate, trifoliate (*Medicago*, *Trifolium*), sometimes phyllodic (many species of *Acacia*), or reduced to a tendril (as in *Lathyrus*), spirally arranged, with stipules present that are sometimes large and leaf-like (*Pisum*) or developed into spines (*Prosopis*, *Robinia*).

Flowers are usually regular or irregular (i.e., actinomorphic to zygomorphic in symmetry, respectively), bisexual, with a single superior carpel (hypogynous to perigynous), pentamerous, arranged singly or in racemes, spikes, or heads. The principal unifying feature of the family is the fruit, the legume [7]. With a few exceptions, legumes are typically one-chambered pods (one locule), with parietal placentation along the adaxial suture, ovules 2 to many, in two alternating rows on a single placenta, typically dry and dehiscent along one or both sutures (legume).

TAXONOMY [8]

Taxonomically, Fabaceae has been traditionally divided into three subfamilies-

- Caesalpinioideae
- Mimosoideae
- Papilionoideae

The recognition of three subfamilies is based on characteristics particularly of the flower, aestivation of petals, sepals (united or free), stamen number and heteromorphy, pollen (single or polyads), leaf complexity, and presence of root nodules. Differences in these characteristics led to the view that the Mimosoideae and Papilionoideae are unique and distinct lineages in the family which arose independently within a paraphyletic "basal" caesalpinioid assemblage.

Agricultural & Economic Importance of Legumes

Legumes have demonstrated agricultural importance for thousands of years, beginning with the domestication of lentils (*Lens esculenta*) in Iran dating to 9,500 to 8,000 years ago, their use as a food source during the prehistory of North and South America (beans, more than 3,000 years ago), and their use by the Roman Empire as a food source and for soil improvement [9]. Today legumes are an increasingly invaluable food source not just for humans, accounting for 27% of the world's primary crop production, but also for farm animals [9]. Legumes were grown on more than 13% of the total arable land under cultivation in the world in 2004 [14]. Grain legumes alone contribute 33% of the dietary protein nitrogen needs of humans, while soybeans (*Glycine max*) and peanut (*Arachis hypogeae*) provide more than 35% of the world's processed vegetable oil and a rich source of dietary protein for the poultry and pork industries [9].

While they produce nitrogen-containing protein in abundance, legumes are deficient in sulfur containing amino acids and other nutrients needed by people and animals. For this reason, legumes and cereal crops are often raised together to account for the amino acids and other elements they are each deficient in [10]. The primary dietary legumes grown, such as bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), chickpea (*Cicer arietinum*), broad bean (*Vicia faba*), pigeon pea (*Cajanus cajan*), cowpea (*Vigna unguiculata*), and lentils (Graham and Vance, 2003), include representatives of each of the four clades within papilionoids, the genistoids, dalbergioids, Hologalegina, and phaseoloid/millettioids.

Industrially, legumes have many uses in making biodegradable plastics, oils, dyes, and biodiesel fuel. Legumes are used traditionally in folk medicines, but also demonstrate importance in modern medicine. Isoflavones commonly found in legumes are thought to reduce the risk of cancer and lower cholesterol and soybean phytoestrogens are being studied for use in postmenopausal hormone replacement therapy (Graham and Vance, 2003). Legumes also produce a hypoglycemic effect when eaten, making them a recommended food for diabetics [10].

2. The plant: Acacia nilotica [11-14]

Acacia nilotica is also known as Gum Arabic tree, Babul, Egyptian thorn, or Prickly Acacia is multipurpose nitrogen fixing tree legume. It occurs from sea level to over 2000 m and withstand at extreme temperature (>50^{o} C) and air dryness. It is widely

spread in subtropical and tropical Africa from Egypt to Mauritania southwards to South Africa, and in Asia eastwards to Pakistan and India.

3. Synonyms:
ACAR11: Acacia arabica (Lam.) Willd
MINI2 : Mimosa nilotica L

4. Taxonomical classification
Kingdom Plantae – Plants
Subkingdom: *Tracheobionta* – Vascular plants
Superdivision :*Spermatophyta* – Seed plants
Division: *Magnoliophyta* – Flowering plants
Class: *Magnoliopsida* – Dicotyledons
Subclass: *Rosidae*
Order: *Fabales*
Family :*Fabaceae* – Pea family
Genus: *Acacia* Mill. – acacia
Species: *Acacia nilotica* (L.) Willd. ex Delile – gum arabic tree

5. Plant Description
Acacia nilotica is a single stemmed plant with a well-developed deep root system.

Height
The average height of the plant has been 15-18 m in height and 2-3 m in diameter.

Pods and Seeds
Pods are 7-15 cm long, green and tomentose (when immature) or greenish black (when mature), indehiscent, deeply constricted between the seed giving a necklace appearance. Seeds are 8-12 per pod, compressed, ovoid, dark brown shining with hard testa [15].

Leaves
The leaves are bipinnate, pinnate 3-10 pairs, 1.3- 3.8 cm long, leaflets 10-20 pairs, and 2-5mm long [16]

Flowers
Flowers are globular heads, 1.2-1.5 cm in diameter of a bright golden yellow color, develop either in axillary or whorl pattern on peduncles 2-3 cm long located at the end of branches [17]

Stem
Stems are usually dark to black colored, deep longitudinal fissured, grey-pinkish slash, exuding a reddish low quality gum [17].

Bark
The bark a tinge of orange and/or green (young tree), but older trees have dark, rough bark and tend to lose their thorns [18].

Thorns
Thorns are thin, straight, light grey exist in axillary pairs (usually 3-12), 5-7.5 cm long in young trees.

Root
Root is generally of brown color in older and whitish in younger regions.

Gum
The gum varies in color from very pale yellowish brown to dark reddish brown depending on the quantity of tannins in the sample. The lighter, more highly valued gums are soluble in water and very viscous; the tannins in the darker gum reduce the solubility. The gum has a moisture content of about 13% and is slightly dextrorotary [19].

6. Growth pattern and germination
Acacia nilotica is a tropical species found throughout India and occurs from sea-level to over 2000 m altitude. Prickly Acacia germinates in rainfall in the wet season. But some seeds may still germinate up to 15 years after seed drop. Seedlings grow rapidly near water but more slowly in open grasslands. It grows in average annual temperatures range from 15–28°C, being frost sensitive when young and withstanding daily maximum temperatures of 50°C [20]. The mean maximum temperature of the hottest month is 25–42ºC and the mean minimum temperature of the coldest month 6–23°C. Babul plant prefers dry conditions, with an annual rainfall of (100–) 250–1500(–2300) mm. This subspecies is commonly found on soils with high clay content, but may grow on deep sandy loam in areas of higher rainfall. It commonly grows close to waterways on seasonally flooded river flats and tolerates salinity well [21]. Trees can flower and fruit two to three years after germination, but after high rainfall it is more quickly, usually between March and June [22]. Pods are formed between July and December. Most leaf fall between June and November and seed pods drop during October to January [23]. Seeds are very simple. Inner integument degenerates completely and the testa is formed by the outer integument [24, 25]. Meharia (2005) has observed that A. nilotica is more productive than A. tortilis after slat treatment. It grows well in two types of soils i.e. riverian alluvial soil and black cotton soil [26].

DISTRIBUTION
The native distribution of *Acacia nilotica* includes much of Africa and the Indian subcontinent [13]. From the GRIN database, the native distribution includes [27].

Africa: Algeria, Angola, Botswana, Egypt, Ethiopia, Gambia, Ghana, Guinea-Bissau, Kenya, Libya, Malawi, Mali, Mozambique, Niger, Nigeria, Senegal, Somalia, South Africa, Sudan, Tanzania, Togo, Uganda, Zambia, Zimbabwe

Asia: Iran, Iraq, Israel, Oman, Saudi Arabia, Syria, Yemen, India, Nepal, Pakistan.

In Bangladesh it is found in Bogra, Faridpur, Jessore, Kushtia, Pabna, Rajshahi also planted by the road sides and embankments throughout the country.

Table 1: Some common medicinal uses of different parts of *Acacia nilotica*

Part used	Uses
Root	The roots are used against cancers and/or tumors (of ear, eye, or testicles), tuberculosis and indurations of liver and spleen [28].
Leaf	Chemoprventive, anitmutagenic, anti-bacterial, anticancer, astringent, anti-microbial activity Tender leaves are used to treat diarrhea, Aphrodisiac, dressing of ulcers, anti-inflammatory and Alzheimer's diseases [29].
Gum	Astringent, emollient, liver tonic, antipyretic and antiasthmatic [30].
Stem bark	Anti-bacterial, antioxidant, anti-mutagenic, cytotoxic bark is used as astringent, acrid cooling, styptic, emollient, anthelmintic, aphrodisiac, diuretic, expectorant, emetic, nutritive, in hemorrhage, wound ulcers, leprosy, leucoderma, small pox, skin diseases, biliousness, burning sensation, toothache, leucoderma, dysentery and seminal weakness. The trunk bark is used for cold, bronchitis, diarrhoea, dysentery, biliousness, bleeding piles and leucoderma [31].
Seeds	Spasmogenic activity and antiplasmodial activity [32].
Pods	Anti-hypertensive and antispasmodic, anti-diarrhoeal, astringent, anti-fertility and against HIV-1 PR, Inhibited HIV-1 induced cythopathogenicity, antiplatelet aggregatory activity and anti-oxidant [33].

Fig 1: Flower of *Acacia nilotica*

Fig 2: Bark of *Acacia nilotica*

Fig 3: Whole tree of *Acacia nilotica*

MATERIALS FOR PARTITIONING AND EXTRACT PREPARATION

Glass wares

Table 2: List of glass wares

Materials	Source
Distilled machine	BDH Laboratory Equipments
Conical flasks (250 ml)	BDH Laboratory Equipments
Beakers (100 ml, 500 ml)	BDH Laboratory Equipments
Test tubes	BDH Laboratory Equipments
Funnels	BDH Laboratory Equipments
Measuring cylinders	BDH Laboratory Equipments
Pipettes	BDH Laboratory Equipments
Automatic pipette puller	Bel-Art Products, USA

Solvents

Table 3: List of solvents

Materials	Source
n-Hexane	Merck
Carbon tetrachloride (CCl_4)	Merck
Dichloromethane (CH_2Cl_2)	Merck
Ethyl acetate ($CH_2CH_3OOCCH_3$)	Merck
Methanol	Scharlau
Acetic acid	Merck
Ethanol	Merck
Distilled Water	-

Filter aid

Table 4: List of filter aid

Filter aids
Filter Paper (Whatman no. 1)
Normal Cotton

Equipments

Table 5: List of equipments

Equipments	Source
Rotary vacuum evaporator	-
Electronic balance	Denver Instruments M-220
Table-top UV detector (252 & 366	*CAMAG*
Grinding machine	-
Oven (0^0C-210^0C)	Gallen Kamp Hotbox
Solvent distillation plant	University Instruments Lab
Distilled water plant	University Instruments Lab

Collection and preparation of plant material

Plant sample (bark) of *Acacia nilotica* was collected from Pabna, Bangladesh in April 2012. Then proper identification of plant sample was done by an expert taxonomist. The bark was sun dried for several days. The plant materials were then oven dried for 24 hours at considerably low temperature for better grinding. The dried bark was then ground in coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Faculty of Pharmacy; University of Dhaka.

Extraction of the Plant Material

About 950 gm of the powdered material was taken in separate clean, round bottomed flask (4.5 liters) and soaked in 5 liter of methanol. The container with its content was sealed by cotton plug and aluminum foil and kept for a period of 21 days accompanying occasional shaking and stirring. The whole mixture was then filtered through cotton followed by Whatman No.1 filter paper and the filtrate thus obtained was concentrated at 39ºC with a Heidolph rotary evaporation. The concentrated extract was then air dried to solid residue. The weight of the crude methanol extract obtained from the powdered whole plant was 22 gm.

Solvent-solvent partition of crude extract

Solvent-solvent partitioning of crude methanolic extract was done following Modified Kupchan Partition [34].

Preparation of mother solution

5 gm of methanol extract was triturated with 90 ml of methanol containing 10 ml of distilled water. The crude extract was dissolved completely. This was the mother solution which was partitioned off successively by four solvents of different polarity. In subsequent stages each of the fractions was analyzed separately for the detection and identification of compounds having antibacterial, cytotoxic, antioxidant and other pharmacological properties.

Partition with n-hexane

The mother solution was taken in a separating funnel. 100 ml of the n-hexane was added to it and the funnel was shaken and then kept undisturbed. The organic portion was collected. The process was repeated thrice (100 ml × 3). The n-hexane fraction was then air dried for solid residue.

Partition with carbon tetrachloride

To the mother solution left after partitioning with n-hexane; 12.5 ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with carbon tetrachloride (CCl_4). The process was repeated thrice (100 ml × 3). The carbon tetrachloride fraction was then air dried for solid residue. The aqueous fraction was preserved for the next step.

Partition with dichloromethane

To the mother solution that was left after partitioning with petroleum ether and carbon tetrachloride; 16 ml of distilled water was added and mixed uniformly. The mother solution was then taken in a separating funnel and extracted with dichloromethane (CH_2Cl_2) (100 ml × 3). The dichloromethane soluble fractions were collected together and air dried. The aqueous fraction was preserved for the next step.

Partition with ethyl acetate

To the mother solution that was left after washing with petroleum ether, carbon tetrachloride and dichloromethane; was then taken in a separating funnel and extracted with ethyl acetate (100 ml × 3). The ethyl acetate soluble fractions were collected together and air dried.

Flow Diagram

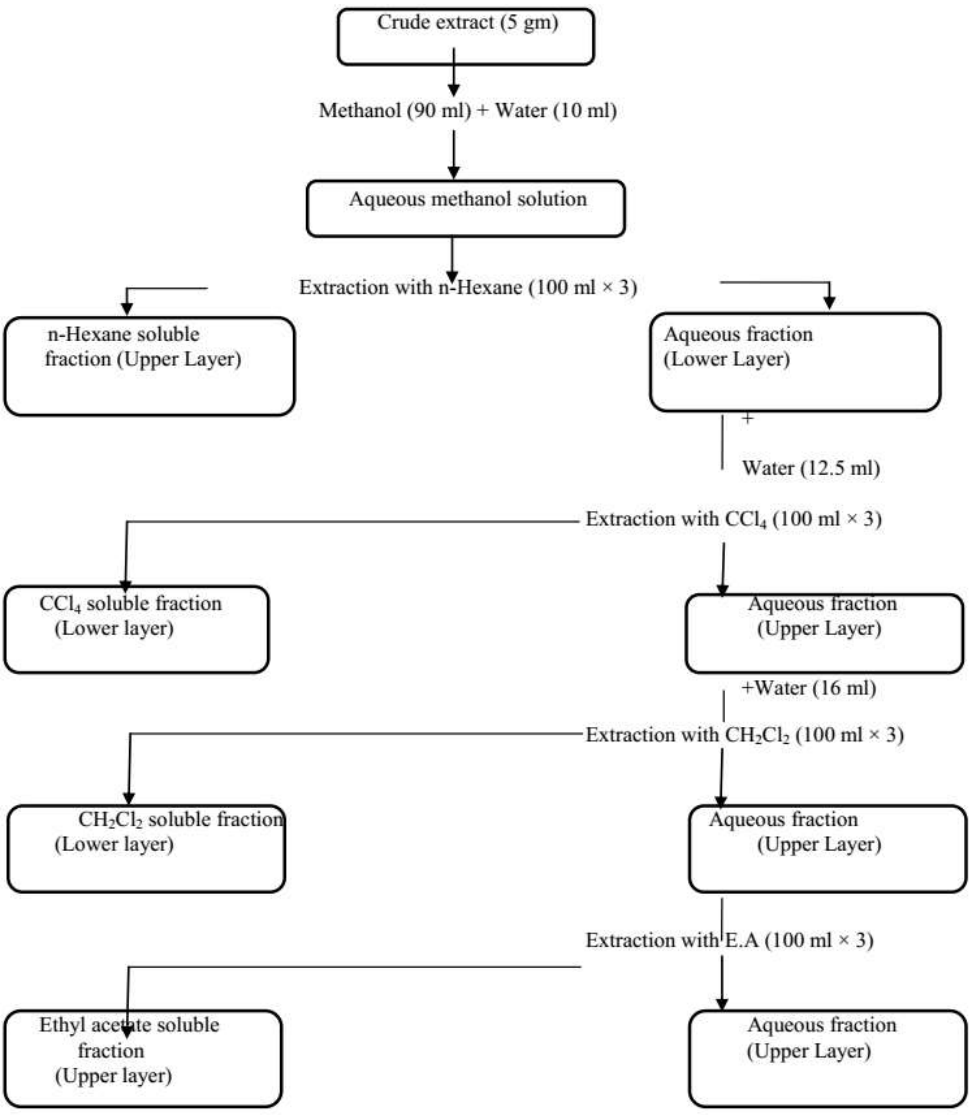

Fig 4: Schematic representation of the modified Kupchan Partitioning of methanolic crude extract of *Acacia nilotica.*

After evaporation the weight of the different fractions obtained are as follows:

Table 6: Amount of fractions after fractionation of crude methanolic extract

Fraction	Weight
n-Hexane soluble fraction	0.50 g
Carbon tetrachloride soluble fraction	0.90 g
Dichloromethane soluble fraction	1.25 g
Ethyl acetate soluble fraction	1.80 g

PRINCIPLE OF ACETIC ACID INDUCED WRITHING METHOD

In this method acetic acid is administered intra-peritoneal to the experimental animals to create pain sensation. As a result, the animals squirms their body at regular interval out of pain. This squirm or contraction of the body is termed as "writhing". As long as the animals feel pain, they continue to give writhing. Each writhing is counted and taken as an indication of pain sensation. Any substance that has got analgesic activity is supposed to lessen the number of writhing of animals within in a given time frame and with respect to

the control group. The writhing inhibition of positive control was taken as standard and compared with test samples and control. As positive control, any standard NSAID drug can be used. In the present study, Diclofenac Na was used to serve the purpose [35-36].

EXPERIMENTAL ANIMALS

Swiss-albino mice of either sex, aged 4-5 weeks, weighing 20-25 gm were collected from Jahangirnagar University animal house. They were kept in clean and only dry polypropylene cages with 12 hours light dark cycle at 25+2°C and 45-55% relative humidity. The animals were fed with pelletized mice feed supplied from ICDDR, B.

EXPERIMENTAL DESIGN

Twenty eight experimental animals were randomly selected and divided into seven groups denoted as group-I, group-II, group-III, group- lV, group-V, group-VI and group-VII consisting of four mice in each group. Each group received a particular treatment i.e. control, standard and dose of the extract of different fractions of bark fraction of the plant respectively. Prior to any treatment, each mouse was weighed properly and the doses of the test samples, standard and control materials were adjusted accordingly.

METHOD OF IDENTIFICATION OF ANIMALS

Each group consisted of four mice. As it was difficult to observe the biologic response of four mice at a time receiving same treatment, it was quite necessary to identify individual animal of a group during the treatment. The animals were individualized by marked as M-1=Mice 1, M-2=Mice 2, M-3=Mice 3, M-4=Mice 4.

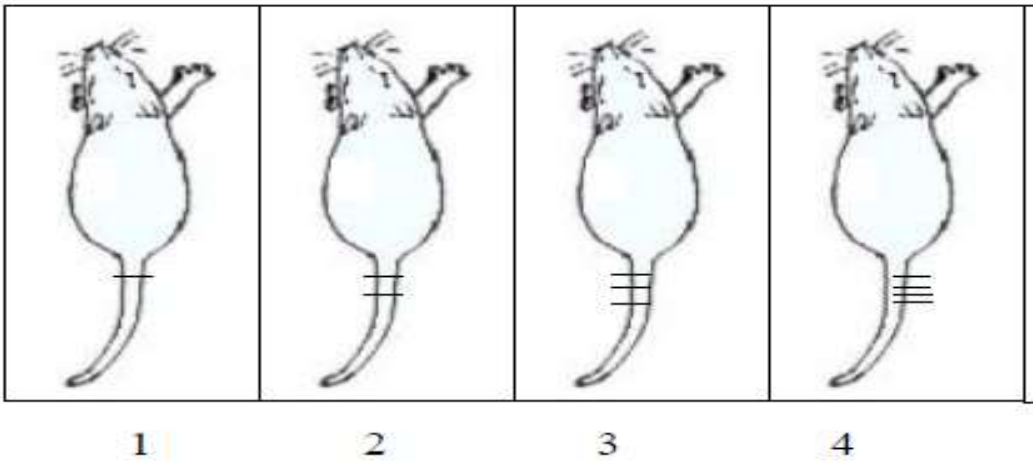

Fig 5: **Identification of test animal**

PREPARATION OF TEST SAMPLES

In order to administer the crude extract or portioning fractions at doses of 400 mg/kg body weight 100 mg of the extract/fraction were measured and triturated unidirectional way by the addition of small amount of Tween-80 (a suspending agent). After proper mixing of extract and suspending agent, normal saline was slowly added. The final volume of the suspension was made up to 3.0 ml. To stabilize the suspension, it was stirred well by vortex mixture.

PREPARATION OF STANDARD SAMPLE

For the preparation of Diclofenac Na at the dose of 10-mg/kg-body weight, 5 mg of Diclofenac Na was taken and a suspension of 5 ml was made.

PREPARATION OF CONTROL SAMPLE

Tween-80 (1%) and DMSO are mixed properly in the normal saline and the volume was made up to 3 ml.

PREPARATION OF ACETIC ACID SOLUTION

For the preparation of acetic acid solution, 0.7 ml acetic acid was diluted with 100 ml distilled water.

Table 7: Test samples used in the evaluation of analgesic effect of crude extract and different fractions of bark of *Acacia nilotica*

Test samples	Group	Purpose	Dose (mg/kg)	Route of administration
1%Tween 80 & DMSO in saline	I	Control Group	0.15 ml/10 gm of body weight	Oral
Diclofenac Na	II	Standard Group	10	Oral
Crude Extract	III	Test sample	400	Oral
EAAN	IV	Test sample	400	Oral
DCMAN	V	Test sample	400	Oral
CTAN	VI	Test sample	400	Oral
HXAN	VII	Test sample	400	Oral
Acetic acid	All	Writhing inducer	0.1 ml /10gm body weight	Intraperitoneal

BE = Bark Extract, DCM = Dichloro Methane, CCl_4 = Carbon Tetrachloride, EA=Ethyl acetate

PROCEDURE

- The animals were weighed and randomly divided into seven groups consisting of 4 mice in each group.
- At zero hour test samples, control (1% Tween-80 solution in saline) and Diclofenac Na were administered orally by means of a long needle with a ball-shaped end.
- Among the mice, group I was considered as control receiving 0.3 ml Tween 80 and DMSO in normal saline each, group II was considered as standard receiving 0.2ml Diclofenac Na each, group III-VII was kept as test groups receiving bark crude extract 0.3 ml each, ethyl acetate fraction 0.3 ml each, dichloromethane fraction 0.3 ml each, carbon tetrachloride fraction 0.3 ml each, hexane fraction 0.3 ml each respectively.
- After 40 minutes acetic acid (0.7%) was administered intra-peritoneal to each of the animals of all the groups
- The forty minutes interval between the oral administration of test materials and intra-peritoneal administration of acetic acid was given to assure proper absorption of the administered samples.
- Five minutes after the administration of acetic acid, number of squirms or writhing were counted for each mouse for fifteen minutes.
- Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly two half writhing were taken as one full writhing.
- Then the number of writhing of the test sample was compared in relative to control and standard groups.

MECHANISM OF PAIN INDUCTION IN ACETIC ACID INDUCED WRITHING [37]

Intraperitoneal administration of acetic acid (0.7%) causes localized inflammation in mice. Following inflammation, there is biogenesis of prostaglandins (from cyclooxygenase pathway) and leukotrienes (lipooxygenase pathway). The released prostaglandins, mainly prostacyclin (PGI_2) and prostaglandin-E have been reported responsible for pain sensation.

The exact mechanisms by which prostaglandins produce pain are not still clear but there are a number of proposed mechanisms of action:

1. All kinds of pain or noxious stimuli (nociception) are conveyed by specific nerves called un-myelinated C fibers and myelinated A-δ fibers, the former being slow conducting and the latter being fast conducting. It has been investigated that un-myelinated C fibers are the most usual conveyer of two.
2. The prostaglandin and other liberated products of inflammation serve as noxious stimuli. They are supposed to sensitize C fibers and subsequently reduce pain threshold. The C fibers get stimulated and cause enhanced release of tachy Kinins, mainly substance P and neuro Kinins. It is the substance P released in excessive amount following the stimulation of C fibers that has been held responsible for sensation of pain in animal.
3. The precise mechanism by which substance P arouses pain sensation is not well documented. But upon release, substance P and other tachy Kinins bind to specific receptors (NK_1, NK_2, NK_3) that are G-protein coupled. Among these receptors, substance P is specifically bound to NK. After binding of substance P to the respective receptor, there is stimulation of

4. Phospholipase C resulting in the formation of two second messengers-Inositol triphosphate (IP_3) and Diacylglycerol (DAG). IP_3 causes the exocytotic release of Ca^{++} stored intra-cellularly and DAG activates protein kinase C which then causes the influx of Ca^{++} through the voltage gated Ca^{++} channel. These happenings may have role in the neural processing of the pain sensation and its subsequent conveyance to higher centers of the brain.
5. Prostaglandins also potentiate the pain producing activity of bradykinins and other autacoids (Rang, 2003)

(A) Housing of test animal

(B) Oral administration of test sample and

(C) Intraperitoneal administration of acetic acid

(D) Writing effect of mice

Fig 6: (A) Housing of test animal, (B) Oral administration of test sample and (C) Intraperitoneal administration of acetic acid (D) Writing effect of mice

RESULTS

The Methanolic extract of *Acacia nilotica* bark and its different solvent soluble fractions were subjected to screening for analgesic activity by acetic acid induced writhing inhibition method. The test was performed by taking crude Methanolic extracts and other partitioning fractions at dose of 400mg/kg body weight.

Table 8: Screening of the analgesic activity by counting the number of writhing after the intraperitoneal administration of 0.7% acetic acid

Animal group	Writhing Count				Average	Writhing %	Inhibition %
	M1	M2	M3	M4			
CL	20	24	18	22	21	100.0	0
STD	6	7	11	10	8.5	40.5	60.5
Crude	13	14	17	11	13.75	65.5	36.0
EAF	16	15	17	14	15.5	73.8	27.9
CCl_4F	13	14	15	12	13.5	64.3	37.2
HexF	13	17	17	14	15.25	72.6	29.1
DCMF	13	10	15	14	13	61.9	39.5

Cl= Control, STD= Standard, EA= Ethyl acetate fraction, CCl_4F= Carbon tetrachloride fraction, HexF= Hexane fraction, DCMF= Dichloromethane fraction.

Table 9: Analgesic activity of Methanolic crude extract and its different fractions of *Acacia nilotica* bark

Animal group	Number of writhing (mean + SEM)	Inhibition %
Control	21 + 1.29	0
STD	8.5 + 1.25	60.5
MEAN	13.75 + 1.19	36.0
EAAN	15.5 + 0.65	27.9
CTAN	13.5 + 0.65	37.2
HXAN	15.25 + 1.03	29.1
DMAN	13 + 1.08	39.5

Note: Each value represents the mean ± SEM., N= 4.

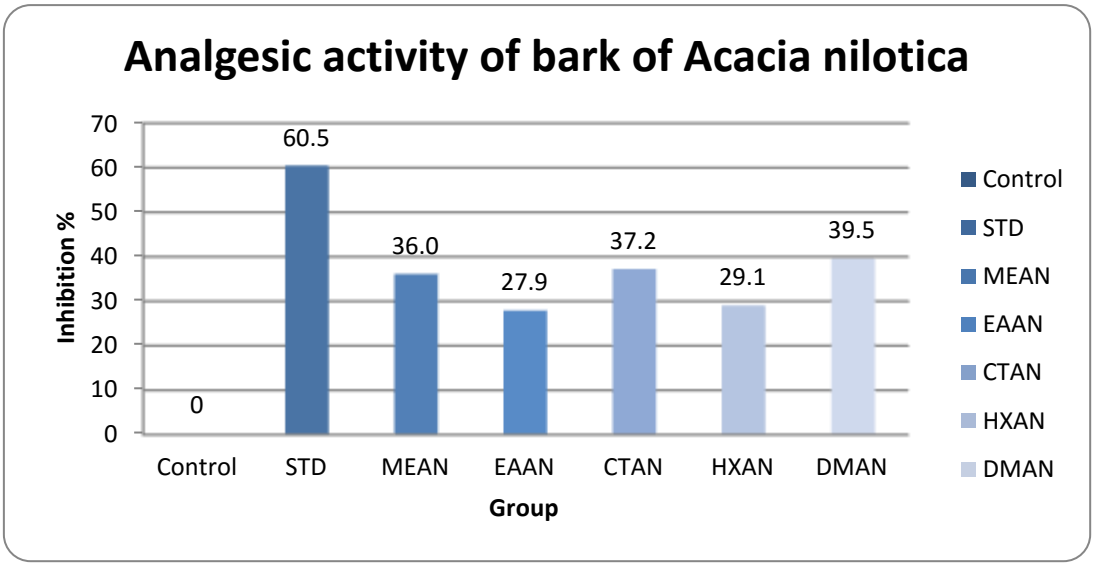

Fig 7: Analgesic activity of Methanolic crude extracts and its different partitioning fraction of bark of *Acacia nilotica*.

STD= Standard, EAAN= Ethyl acetate fraction of *Acacia nilotica*, CTAN= Carbon tetrachloride fraction of *Acacia nilotica*, HXAN= Hexane fraction of *Acacia nilotica*, DMAN= Dichloromethane fraction of *Acacia nilotica*.

DISCUSSION

The crude Methanolic extract of bark of *Acacia nilotica* at the dose of 400 mg/kg body weight have mild decrease in the number of writhes (36% of inhibition) when compared to the control untreated group (Table 9).The partitioning fractions such as Ethyl acetate fraction, Carbon tetrachloride fraction, Hexane fraction, Dichloromethane fraction soluble fractions at the same dose exhibited a lower decrease in the number of writhes (27.9, 37.2, 29.1, 39.5 % inhibition respectively).

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