ANTI-INFLAMMATORYAND DIURETIC ACTIVITY OFDESMODIUM MOTORIUM LEAVES

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ABSTRACT:
Medicinal plants have enormous economic importance on the Indian subcontinent. Nature has given us an incredibly wide variety of plant species, and they can be found growing all around the country. The three categories of biodiversity—species diversity, genetic diversity, and habitat diversity—are all highly prevalent in India. A well-known plant called Desmodium species has been connected to a variety of pharmacological effects. It is a well-known drug that is administered as an infusion or syrup to treat dyspepsia. Ayurveda claims that the drug primarily targets the bladder to treat rheumatism and urinary disorders. Despite the fact that this drug's chemical makeup hasn't been extensively studied and its biological activity hasn't gotten much attention. The anti-inflammatory activity was investigated using a carrageenan-induced hind paw edema model. The anti-inflammatory efficacy of Desmodium motorium extracts (inhibition of edema) was studied using paw volume. The Diuretic activity was screened using LIPSCHITZ test for diuretic activity model. The extracts of Desmodium motorium were tested for diuretic activity. The present study showed that Desmodium motorium significantly increases the urine output. However, the above data only provide lead for further investigation of the pharmacological action of Desmodium motorium in more appropriate models like anaesthetized dogs and isolation of active principles and finding the phytochemical(s) for the diuretic activity and the mechanism of action.
Keywords: Herbs, anti – inflammatory, diuretic

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1.1. GENERAL

Medicinal plants are a source of great economic value in the Indian subcontinent. Nature has best owed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. India is rich in all the 3 levels of biodiversity,

namely species diversity, genetic diversity and habitat diversity. In India thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and fewer side effects. However, the last few years have seen a major increase in their use in the developed world.[1]In developed countries, there is a growing interest in the use of medicinal plants for treating health conditions. However, information is lacking about mechanisms of action and possible differences between related species of plants.[2]

Although herbal medicine use continues to grow in many disease conditions, the risk from use may over shadow potential benefit, especially in the renal compromised patient population. Vulnerable times for the renal compromised patient include predialysis, dialysis, and the post- renal transplant periods. They may also be confronting co-morbid disease states such as hypertension or diabetes. Renal patients may reach for additional therapy in the form of herbal dietary supplements because they experience adverse side effects or lack of efficacy from conventional medicines.[3]

ADVANTAGE:

- Medicinal herbs are used as traditional medicine worldwide as these are cheaper, easily available and their use depends on ancestral experience.
- The World Health Organization encourages the inclusion of herbal medicines proven safe and efficacious in the health care programs of developing countries because of the great potential they have in combating various diseases.
- The medicinal and aromatic plants have been used since ancient times for the treatment of human ailment and as natural food preservatives.
- Plants are one of the most important sources of medicines.

1.1.1. IMPORTANCE OF HERBAL PLANTS

In modern western medicine, approximately 50 % of drugs are in clinical use are derived from natural sources and these halves are plant based. It recognized and considered to be much safe than the synthetic drugs and price wise within the search of common man. Indigenous drugs can definitely open up new vistas in therapy and purified natural compounds may serve as template for synthesis of new generation drugs which in turn may have low toxicity and better therapeutic index.[4] Chemical medicines indeed have a more powerful effect than medicinal herbs, although they present a higher degree of side effects and risks. They are generally less potent than their pure drug relatives because they contain a mixture of many chemicals in small quantities.[7]

Even so, herbal products are not free of risk but, the recent popularity of herbal medicines means that many patients need reliable information on using these substances appropriately. Most botanical products, vitamins, minerals, amino acids and mammalian tissue abstracts are currently regulated only under the federal dietary supplement Health& Education act of 1994. This act limits the FDA's authority to

require proof of efficacy, safety and quality before these products are sold commercially.[8]

1.1.2. BASIS OF SELECTION OF PLANT:

The pharmacological studies in alkaloids have been largely concerned with the effect of alkaloids on physiological processes other than inflammation. The alkaloids tend to be rather toxic, although the toxicity appears to be well below the therapeutic levels. The alkaloids appear to offer the considerable promise for further investigation as anti-inflammatory compounds, and some appears to be remarkably active. Of the 171 evaluated alkaloids, 137 presented anti-inflammatory activity, and among those, the is quinoline type was the most studied. The Carrageenan induced paw edema was the most used model for evaluating the anti-inflammatory activity.[9]it can inhibit inflammatory process mediated by "leukotrienes" hyaluronidase and lysosomal enzymes of localized inflammation. Therefore, it could be assumed that the inhibition of inflammation of Disodium motorium was probably due to alkaloids.[10, 11] The plant contains Flavonoids. As flavonoids have diverse the effects on improvement of moods, protection of deficits, and central nervous system disorder and play major role in stress induce depression. [12],[13.] The phytochemical investigations have shown the presence of flavonoids (flavonol glycosides) in *Desmodium motorium*. Hence, the present work was undertaken to investigate the effect of crude extracts of *Desmodium motorium* in animal models of experimentally induced diuresis. If the results are encouraging or significant; the investigated product will be useful in the management of disorders.

1.1.3. PLANT DESCRIPTION

The Telegraph Plant (*Desmodium motorium*), is a tropical Asian shrub having trifoliate compound leaves, known for its slow movement of small lateral leaflets which rotate on their axes and jerk up and down with a period of about 3 to 5 minutes, under the influence of sunshine. This plant is one of a few plants capable of rapid movement; others include Mimosa and the Venus flytrap.

Scientific classification:

Plant: *Desmodium motorium*
Family: Papilionaceae
Genus: Desmodium
Species: gyrans
Synonym: Codariocalyx motorius, Hedysarum gyran, Desmodium roylei, Codariocalyx gyrans, Hedysarum motorium, Hedysarum gyrans, Meibomia gyrans, Gyred cock's head, move plant, Moving plant, Telegraph plant,
Category: Herbs, Perennials, Shrubs
Bloom Color: This plant produces small purple flowers.
Foliage: Herbaceous

Fig no. 1: Desmodium motorium

Medicinal application:

1. The root is widely used in Indian system of medicine and is being used in fever.
2. Whole plant is also used as emollient, laxative, anti-dysentery, anti-tussive and asthma. Its macerated fresh leaves and fruits are applied externally to wounds. Leaves are also tonic. Roots are considered carminative and used in billions of complaints.
3. Leaves are galactagogue and mixed with cow milk for this use, they are also given to children for diarrhea due to indigestion and also in convulsion. Juice of fresh plant is applied to abscesses and wounds that do not heal readily [14,15].
4. It is used traditionally because of its many pharmacological activities. Juice of fresh plant is applied to abscesses and wounds that do not heal readily.
5. Leaves have febrifugal and tonic properties. Its macerated fresh leaves, fruits and flowers are applied externally to wounds. Leaves are galactagogue and mixed with cow milk for this use, they are also given to children for diarrhea due to indigestion and also in convulsion.
6. Roots used (in Indian medicine) as a remedy for asthma and coughs, as an ant dysenteric, an emollient and a laxative. The root is widely used in Indian system of medicine for rheumatism and is being used in fever. Roots are considered carminative, tonic and used in billions of complaints.

Ayurvedic properties:

1. "Bhunakra", "Ote-atil": Root is used in treating rheumatism. It is also used as aphrodisiac.
2. Leaf and flowers are used for the treatment of wounds. [14, 15, 16]

1.2. INFLAMMATION

Inflammation (Latin, inflammation, to set on fire) is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation is not a synonym for infection. Even in cases where inflammation is caused by infection it is incorrect to use the terms as synonyms: infection is caused by an exogenous pathogen, while inflammation is the response of the organism to the pathogen.[17]

In the absence of inflammation, wounds and infections would never heal and progressive destruction of the tissue would compromise the survival of the organism. However, inflammation which runs unchecked can also lead to a host of diseases, such as hay fever,

atherosclerosis, and rheumatoid arthritis. It is for this reason that inflammation is normally tightly regulated by the body.[18]

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue.[19]

Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.[20]

1.2.1. TYPES OF INFLAMMATION

	Acute	Chronic
Causative agent	Pathogens, injured tissues	Persistent acute inflammation due to non-degradable pathogens, persistent foreign bodies, or autoimmune reactions
Major cells involved	Neutrophils, mononuclear cells (monocytes, macrophages)	Mononuclear cells (monocytes, macrophages, lymphocytes, plasma cells), fibroblasts
Primary mediators	Vasoactive amines, eicosanoids	IFN-γ and other cytokines, growth factors, reactive oxygen species, hydrolytic enzymes
Onset	Immediate	Delayed
Duration	Few days	Up to many months, or years
Outcomes	Healing, abscess formation, chronic inflammation	Tissue destruction, fibrosis

1.2.2. SYMPTOMS OF ACUTE INFLAMMATION

Redness
Heat
Swelling
Pain
Loss of function[21]

- Functio lasea is a bit of an apocryphal notion, as it is not really unique to inflammation and is a characteristic of many disease states.[22]

1.3. INFLAMMATORY DISORDER

Abnormalities associated with inflammation comprise a large, unrelated group of disorders which underlie a variety of human diseases.

A large variety of proteins are involved in inflammation, and any one of them is open to a genetic mutation which impairs or otherwise dysregulates the normal function and expression of that protein.[23]

Examples of disorders associated with inflammation include:

- Asthma
- Autoimmune diseases
- Chronic inflammation
- Chronic prostatitis
- Glomerulonephritis
- Hypersensitivities
- Inflammatory bowel diseases
- Pelvic inflammatory disease
- Reperfusion injury
- Rheumatoid arthritis
- Transplant rejection
- Vasculitis

Allergies

An allergic reaction, formally known as type 1 hypersensitivity, is the result of an inappropriate immune response triggering inflammation. A common example is hay fever, which is caused by a hypersensitive response by skin mast cells to allergens. Pre-sensitized mast cells respond by degranulating, releasing vasoactive chemicals such as histamine. These chemicals propagate an excessive inflammatory response characterized by blood vessel dilation, production of pro-inflammatory molecules, cytokine release, and recruitment of leukocytes. Severe inflammatory response may mature into a systemic response known as anaphylaxis.[24]

Other hypersensitivity reactions (type 2 and type 3) are mediated by antibody reactions and induce inflammation by attracting leukocytes which damage surrounding tissue.

Myopathies

Inflammatory myopathies are caused by the immune system inappropriately attacking components of muscle, leading to signs of muscle inflammation. They may occur in conjunction with other immune disorders, such as systemic sclerosis, and include dermatomyositis, polymyositis, and inclusion body myositis.

Leukocyte defects

Due to the central role of leukocytes in the development and propagation of inflammation, defects in leukocyte function often result in a decreased capacity for inflammatory defence with subsequent vulnerability to infection. Dysfunctional leukocytes may be unable to correctly bind to blood vessels due to surface receptor mutations, digest bacteria (Chediak-Higashi syndrome), or produce microbicides (chronic granulomatous disease). Additionally, diseases affecting the bone marrow may result in abnormal or few leukocytes.[25,26]

1.4. ANTI INFLAMMATORY DRUGS

It refers to the property of a substance or treatment that reduces inflammation. Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids which affect the brain.

Many steroids, specifically glucocorticoids, reduce inflammation or swelling by binding to cortisol receptors. These drugs are often referred to as corticosteroids.

Non-steroidal anti-inflammatory drugs (NSAIDs), alleviate pain by counteracting the cyclooxygenase (COX) enzyme. On its own COX enzyme synthesizes prostaglandins,

creating inflammation. In whole the NSAIDs prevent the prostaglandins from ever being synthesized, reducing or eliminating the pain.

Some common examples of NSAIDs are: ibuprofen, aspirin and naproxen. The newer specific COX-inhibitors although probably sharing a similar mode of action are not classified together with the traditional NSAIDs. [27]

In addition to medical drugs, many herbs have anti-inflammatory qualities, including hyssop, ginger, Turmeric, Arnica montana which contains helenalin, a sesquiterpene lactone, and willow bark, which contains salicylic acid, a substance related to the active ingredient in aspirin.

Canna bichromene, one of the many cannabinoids present in the cannabis plant, has been shown to reduce inflammation.

Most NSAIDs act as non-selective inhibitors of the enzyme cyclooxygenase, inhibiting both the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isoenzymes. Cyclooxygenase catalyses the formation of prostaglandins and thromboxane from arachidonic acid (itself derived from the cellular phospholipid bilayer by phospholipase A2). Prostaglandins act (among other things) as messenger molecules in the process of inflammation.

This mechanism of action was elucidated by John Vane, who later received a Nobel Prize for his work.

1.4.1. TYPES OF ANTI-INFLAMMATORY DRUGS

1. STEROIDAL ANTI-INFLAMMATORY DRUGS

Many steroids, specifically glucocorticoids, reduce inflammation or swelling by binding to cortisol receptors. These drugs are often referred to as corticosteroids.

2. NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

Non-steroidal anti-inflammatory drugs (NSAIDs), alleviate pain by counteracting the cyclooxygenase (COX) enzyme. On its own COX enzyme synthesizes prostaglandins, creating inflammation. In whole the NSAIDs prevent the prostaglandins from ever being synthesized, reducing or eliminating the pain.

Some common examples of NSAIDs are: ibuprofen, aspirin and naproxen. The newer specific COX-inhibitors although probably sharing a similar mode of action are not classified together with the traditional NSAIDs. [28]

1.4.2. ADVERSE EFFECTS

Inflammation often affects the numbers of leukocytes present in the body:

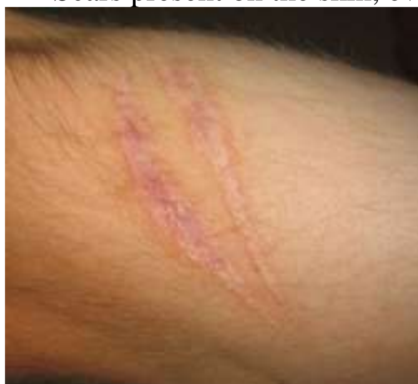
1. Leukocytosis is often seen during inflammation induced by infection, where it results in a large increase in the amount of leukocytes in the blood, especially immature cells. Leukocyte numbers usually increase to between 15 000 and 20 000 cells per ml, but extreme cases can see it approach 100 000 cells per ml. Bacterial infection usually results in an increase of neutrophils, creating neutrophilia, whereas diseases such as asthma, hay fever, and parasite infestation result in an increase in eosinophils, creating eosinophilia.

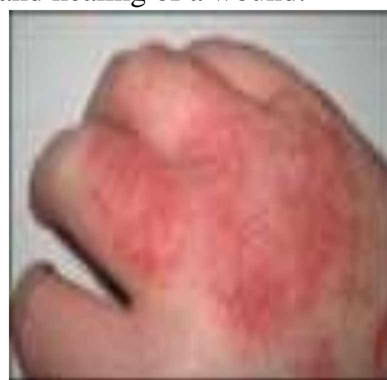
2. Leukopenia can be induced by certain infections and diseases, including viral infection, Rickettsia infection, some protozoa, tuberculosis, and some cancers.[29]

3. Systemic inflammation and obesity

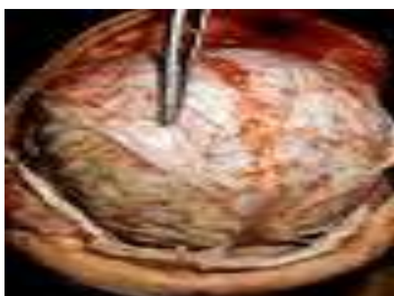
4. Outcomes[30]

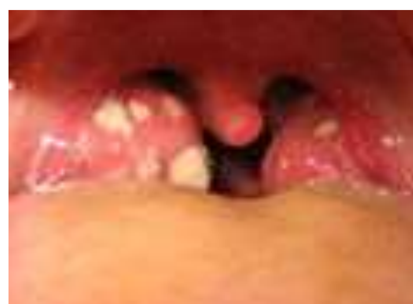
Scars present on the skin, evidence of fibrosis and healing of a wound.





Acute appendicitis **Acute dermatitis**





Acute infective meningitis **Acute tonsillitis**

1.5. DIURETICS: A diuretic is any drug that elevates the rate of urination (diuresis). There are several categories of diuretics. All diuretics increase the excretion of water from the body, although each class of diuretic does so in a distinct way. [31]

High ceiling loop diuretics

High ceiling diuretics are diuretics that may cause a substantial diuresis - up to 20%of the filtered load of NaCl and water. This is huge, compared to that normal. diuretics. Loop diuretics, such as furosemide, inhibit the body's ability to reabsorb sodium at the ascending loop in the kidney which leads to a retention of water in the urine as water normally follows sodium back into the extracellular fluid (ECF). Other examples of high ceiling loop diuretics include ethacrynic acid, torsemide and bumetanide.

Thiazides

Drugs such as hydrochlorothiazide act on the distal tubule and inhibit the Sodium-chloride symporter leading to retention of water in the urine as water normally follows penetrating solutes.

Potassium-sparing diuretics

These are diuretics which do not promote the secretion of potassium into the urine; thus, potassium is spared and not lost as much as in other diuretics. Such drugs include spironolactone which is a competitive antagonist of aldosterone. Aldosterone normally adds sodium channels in the principal cells of the collecting duct and late distal tubule of the nephron. Spironolactone prevents aldosterone from entering the principal cells, preventing sodium reabsorption. Other examples of potassium-sparing diuretics are amiloride, triamterene and spironolactone.[32]

Osmotic diuretics

Compounds such as mannitol are filtered in the glomerulus, but cannot be reabsorbed. Their presence leads to an increase in the osmolarity of the filtrate. To maintain osmotic balance, water is retained in the urine.[33]

High Blood Glucose

Glucose, like mannitol, is a sugar that can behave as an osmotic diuretic. Unlike mannitol, glucose is commonly found in the blood. However, in certain conditions such as diabetes mellitus, the concentration of glucose in the blood exceeds the maximum resorption capacity of the kidney.

When this happens, glucose remains in the filtrate, leading to the osmotic retention of water in the urine. Use of some drugs, especially stimulants may also increase blood glucose and thus increase urination.

1.6. CLASSICATION OF DIURETICS

1. HIGH EFFICACY DIURETICS (INHIBITORS OF Na+-K+-2Cl- CO-TRANSPORT)

a) Sulphonyl derivatives: Furosemide, Bumetanide.

b) Phenoxy acetic acid derivative: Ethacrynic acid.

2. MEDIUM EFFICACY DIURETICS (INHIBITORS OF Na+-Cl- SYMPORT)

a) Benzo thiadiazines (thiazides): Chlorothiazide, Hydrochlorothiazide, Benzthiazide, Hydro flumethiazide, Clopamide.

b) Thiazide like (related heterocyclic): Chlorthalidone, Metolazone, Xipamide, Indipamide.

3. WEAK OR ADJUNCTIVE DIURETICS

a) Carbonic anhydrase inhibitors: Acetazolamide.

b) Potassium sparing diuretics

i. Aldosterone antagonist: Spironolactone.

ii. Directly acting (Inhibitors of renal epithelial Na+ channel): Triamterene, Amiloride.

c) Osmotic diuretics: Mannitol, Isosorbide.

d) Xanthine: Theophylline.[34]

1.7. ADVERSE EFFECTS OF DIURETICS [35]

Hypovolemia
Hyperkalemia
Metabolic alkalosis
Metabolic acidosis
hyperuricemia

1.8. USES OF DIURETICS

1. In medicine, diuretics are used to treat heart failure, liver cirrhosis, hypertension and certain kidney diseases. Some diuretics, such as acetazolamide, help to make the urine more alkaline and are helpful in increasing excretion of substances such as aspirin in cases of overdose or poisoning. Diuretics are often abused by sufferers of eating disorders, especially bulimics, in attempts at weight loss.
2. The antihypertensive actions of some diuretics (thiazides and loop diuretics in particular) are independent of their diuretic effect. That is, the reduction in blood pressure is not due to decreased blood volume resulting from increased urine

production, but occurs through other mechanisms and at lower doses than that required to produce diuresis. [36]

MATERIAL AND METHODS Chemicals and solvents were purchased from Sigma-Aldrich (India), Spectro chem (India), Merck (India). These chemicals, reagents and drugs are used in this formulation. *Desmodium motorium* leaves were collected from Sri Lanka. Diclofenac sodium**,** Frusemide, Dragondroff's reagent, Hager's reagent, Mayer's reagent, Wagner's reagent, Fehling's solution A, Fehling's solution B. Petroleum ether, Chloroform, Ethyl acetate, Ethanol, Methanol

4.2.METHODOLOGY:PHARMACOGNOSTIC INVESTIGATION:

LEAVES COLLECTION AND PLANT AUTHENTICATION:

The leaves of *Desmodium motorium* were collected from Sri Lanka. The plant and leaves were authenticated by Dr. Vishal Saxena, Department of Botany, D.A.V. College, Kanpur. The voucher specimen of the collected sample will be deposited in the institutional herbarium for future reference.

4.2.1.2. EXAMINATION OF POWDER DRUG:

- ORGANOLEPTIC CHARACTERISTIC:

Color: Green
Odor: Musty soil odor
Taste: Bitter

- SOLUBILITY ANALYSIS:

Soluble in: Water, ethyl acetate, ethanol, methanol.
Sparingly soluble in: Chloroform.
Insoluble in: Petroleum ether.[38]

4.2.1.3. POWDER DRUG EVALUATION:

Determination of residue:

The total amount of powder fed in the column before starting the extraction process was 48.7 grams, so the theoretical yield was 48.7 grams.

1. **Calculation for petroleum ether extract:**
   Theoretical yield= 48.7 grams
   Weight of empty china dish=52.43grams
   Weight of china dish + extract=54.83 grams
   Weight of extract= (Weight of china dish + extract) – (Weight of empty china dish)
   = (54.83- 52.43) grams
   =2.4 grams
   Percentage yield= (Practical yield/Theoretical yield) ×100
   = (2.4/48.7) × 100
   = 4.92%
2. **Calculation for chloroform extract:**
   Theoretical yield= 48.7 grams
   Weight of empty china dish=55.64 grams
   Weight of china dish + extract=57.37 grams
   Weight of extract= (Weight of china dish + extract) – (Weight of empty china dish)
   = (57.37-55.64) grams
   =1.73 grams

Percentage yield= (Practical yield/Theoretical yield) ×100
= (1.73/48.7) ×100
=3.55%

3. **Calculation for ethyl acetate extract:**
Theoretical yield= 48.7 grams
Weight of empty china dish=55.95 grams
Weight of china dish + extract=56.65 grams
Weight of extract= (Weight of china dish + extract) – (Weight of empty china dish)
= (56.65-55.95) grams
= 0.7 grams
Percentage yield= (Practical yield/Theoretical yield) ×100
= (0.7/48.7) ×100
=1.43%

4. **Calculation for ethanol extract:**
Theoretical yield= 48.7 grams
Weight of empty china dish= 61.17 grams
Weight of china dish + extract= 62.93 grams
Weight of extract= (Weight of china dish + extract) – (Weight of empty china dish)
= (62.93-61.17) grams
= 1.76 grams
Percentage yield= (Practical yield/Theoretical yield) ×100
= (1.76/48.7) ×100
=3.61%

5. **Calculation for methanol extract:**
Theoretical yield= 48.7 grams
Weight of empty china dish= 53.98 grams
Weight of china dish + extract= 55.50 grams
Weight of extract= (Weight of china dish + extract) – (Weight of empty china dish)
= (55.50-53.98) grams
= 1.52 grams
Percentage yield= (Practical yield/Theoretical yield) ×100
= (1.52/48.7) ×100
=3.12%

6. **Calculation for chloroform water extract:**
Theoretical yield= 48.7 grams
Weight of empty china dish=55.90 grams
Weight of china dish + extract=58.20 grams
Weight of extract= (Weight of china dish + extract) – (Weight of empty china dish)
= (58.20-55.90) grams
= 2.3 grams
Percentage yield= (Practical yield/Theoretical yield) ×100
= (2.3/48.7) ×100
=4.7%

7. **Calculation for alkaloid extract:**
   Theoretical yield= 48.7 grams
   Weight of empty china dish= 52.30 grams
   Weight of china dish + extract= 55.80 grams
   Weight of extract= (Weight of china dish + extract) – (Weight of empty china dish)
   = (55.80-52.30) grams
   = 3.5 grams
   Percentage yield= (Practical yield/Theoretical yield) ×100
   = (3.5/48.7) ×100
   =7.2%
8. **Calculation for aqueous extract:**
   Theoretical yield= 48.7 grams
   Weight of empty china dish= 55.64 grams
   Weight of china dish + extract= 59.23 grams
   Weight of extract= (Weight of china dish + extract) – (Weight of empty china dish)
   = (59.23-55.64) grams
   = 3.59 grams
   Percentage yield= (Practical yield/Theoretical yield) ×100
   = (3.59/48.7) ×100
   =7.34%

4.2.1.4. PREPARATION OF EXTRACT:

The raw material i.e. the leaves of *Desmodium motorium* were washed with running water.

↓

The leaves are then air dried.
(Inshade)

↓

The leaves are then ground.
(Maximum particle size 0.4mm)

↓

The ground sample of dry leaves (50 grams) was extracted with petroleum ether (700ml) up to 72 cycles.

↓

followed by successive extraction through different solvents i.e. after petroleum ether (700ml); chloroform (650ml), ethyl acetate (775ml), ethanol (800ml), methanol (800ml).

↓

Alkaloid extract, chloroform water extract and aqueous extract were also obtained from the same powder by using Soxhlet apparatus.

↓

Each extract was concentrated by distilling off the solvent.

↓

And evaporated till it dries on water bath.

↓

Thus, the crude extracts were obtained.

4.2.1.5. PHYTOCHEMICAL TEST:

The freshly prepared extracts were analyzed for phytochemical constituents as described for the detection of alkaloids, saponins, cardiac glycosides, reducing sugars, flavonoids and tannins. These are described as follows:

1) Tests for Alkaloids:

a) Dragondroff's test: To 2-3 ml filtrate added few drops Dragondroff's reagent and was observed for orange brown precipitate.

b) Mayer's test: 2-3 ml filtrate with few drops Mayer's reagent was observed for Precipitate.

c) Hager's test: 2-3 ml filtrate with Hager's reagent was observed for yellow precipitate.

d) Wagner's test: 2-3 ml filtrate with few drops of Wagner's reagent was observed for reddish brown precipitate.

TABLE:

For the detection of alkaloids:

	Pet. Ether	Chloroform	Ethyl acetate	Ethanol	Methanol	Chloroform water
Dragondroff's reagent	✓	✓	✓	✓	✓	✓
Mayer's reagent	✓	✓	✓	✓	✓	✓
Hager's reagent	✓	✓	✓	✓	✓	✓
Wagner's reagent	✓	✓	✓	✓	✓	✓

2) Tests for Saponin Glycosides: - Foam test: The drug extract was shaken vigorously with water. Persistent foam was observed.

TABLE:

For the detection of saponin glycosides:

	Pet. Ether	Chloroform	Ethyl acetate	Ethanol	Methanol	Chloroform water
Foam Test	✓	✓	✓	✓	✓	✓

3) Tests for Glycosides:

General test for Glycosides:

Part A: To 2-3 ml of extract dil. H_2SO_4 was added and heated on a water bath for 1-2 minutes. Neutralize with 10% NaOH, check with litmus paper and to resulting solution add Fehling's A & B. Increased red precipitate in this case shows glycosides are present.

TABLE:

For the detection of glycosides:

	Pet. Ether	Chloroform	Ethyl acetate	Ethanol	Methanol	Chloroform water

Part A	✓	✓	✓	✓	✓	✓

4) Tests for Flavonoids:

a) Shinoda test: To dried powder or extract, added 5 ml 95% ethanol, few drops concentrated HCl and 0.5 gm magnesium turnings. Pink color was observed.

b) To small quantity of residue, added lead acetate solution observed for Yellow colored precipitate.

c) Addition of increasing amount of sodium hydroxide to the residue was observed as to whether it showed yellow coloration, which was decolorized after addition of acid.

d) Ferric chloride test: To test solution, added few drops of ferric chloride solution observed for intense green color.

TABLE:

For the detection of flavonoids:

	Pet. Ether	Chloroform	Ethyl acetate	Ethanol	Methanol	Chloroform water
Shinoda test	✓	✓	✓	✓	✓	✓
Lead acetate solution	✓	✓	✓	✓	✓	✓
Sodium hydroxide sol.	✓	✓	✓	✓	✓	✓
Ferric chloride test	✓	✓	✓	✓	✓	✓

6) Tests for Steroids:

a) **Salkowski Reaction**: To 2 ml of extract, 2 ml chloroform and 2 ml concentrated H2SO4 was added. Shook well, whether chloroform layer appeared red and acid layer showed greenish yellow fluorescence was observed.

b) **Sulphur powder test:** Add small amount of Sulphur powder to the test solution, it sinks at the bottom.

TABLE:

For the detection of steroids:

	Pet. Ether	Chloroform	Ethyl acetate	Ethanol	Methanol	Chloroform water
Salkowski reaction	✓	✓	Not present	✓	Not present	✓
Sulphur powder test	✓	✓	✓	✓	✓	✓

4.2.1.6. CHROMATOGRAGHY:

4.2.1.6.1. CHROMATOGRAPHY FOR ALKALOIDS:

THIN LAYER CHROMATOGRAPHY

Solvent system: Toluene: Ethyl acetate: Diethylamine (70:20:10)
Detecting agent: Dragondroff's reagent

METHOD

- **Plate preparation:**

1) Plates are prepared by mixing the adsorbent, such as silica gel, with a small amount of inert binder like calcium sulfate (gypsum) and water.
2) This mixture is spread as thick slurry on an unreactive carrier sheet, glass.
3) The plate is dried and activated by heating in an oven for 30 minutes at 110 °C.
4) The thickness of the absorbent layer is typically around 0.1 – 0.25 mm for analytical purposes and around 0.5 – 2.0 mm for preparative TLC.

- **Technique:**

1) A small spot of solution containing the extract is applied to a plate, about 1.5 centimeters from the bottom edge. The solvent is allowed to completely evaporate off, otherwise a very poor or no separation will be achieved.
2) A small amount of an appropriate solvent (eluent) is poured into a glass beaker (separation chamber) to a depth of less than 1 centimeter.
3) The TLC plate is then placed in the chamber so that the spot(s) of the sample do not touch the surface of the eluent in the chamber, and the lid is closed. The solvent moves up the plate by capillary action, meets the sample mixture and carries it up the plate (elutes the sample). The plate should be removed from the chamber before the solvent front reaches the top of the stationary phase (continuation of the elution will give a misleading result) and dried.

- **Separation process:**

1) Separation of compounds is based on the competition of the solute and the mobile phase for binding places on the stationary phase.
2) Silica gel is used as the stationary phase it can be considered polar.
3) Given two compounds which differ in polarity, the more polar compound has a stronger interaction with the silica and is therefore more capable to dispel the mobile phase from the binding places.
4) Consequently, the less polar compound moves higher up the plate (resulting in a higher Rf value).

- **Analysis:**

Specific color reagents exist into which the TLC plate is dipped or which are sprayed onto the plate.

CALCULATION OF Rf VALUES:

$$\text{Rf Value} = \frac{\text{Distance from Baseline travelled by Solute}}{\text{Distance from Baseline travelled by Solvent (Solvent Front)}}$$

a) Petroleum ether:

Spot 1: R_f = 1.8 cm/ 7.3 cm = 0.246

Spot 2: R_f = 5.3 cm/ 7.3 cm = 0.726

Spot 3: R_f = 6.4 cm/ 7.3 cm = 0.876

b) Chloroform:

Spot 1: R_f = 2.7 cm/ 7.1 cm = 0.380

Spot 2: R_f = 6.8 cm/ 7.1 cm = 0.957

Spot 3: R_f = 3.9 cm/ 7.1 cm = 0.549

c) Ethyl acetate:

Spot 1: R_f = 7 cm/ 7.4 cm = 0.945

Spot 2: R_f = 6.6 cm/ 7.4 cm = 0.891

Spot 3: R_f = 5.2 cm/ 7.4 cm = 0.702

d) Ethanol:

Spot 1: R_f = 7.2 cm/ 7.4 cm = 0.972
Spot 2: R_f = 5.9 cm/ 7.4 cm = 0.797
Spot 3: R_f = 5.7 cm/ 7.4 cm = 0.77

e) Methanol:

Spot 1: R_f = 5.6 cm/ 7.2 cm = 0.778
Spot 2: R_f = 6.8 cm/ 7.2 cm = 0.944
Spot 3: R_f = 1.5 cm/ 7.2 cm = 0.208

f) Chloroform water:

Spot 1: R_f = 6.4 cm/ 7.1 cm = 0.901
Spot 2: R_f = 3.7 cm/ 7.1 cm = 0.521
Spot 3: R_f = 3.2 cm/ 7.1 cm = 0.45

4.2.1.6.2. CHROMATOGRAPHY FOR FLAVONOIDS:

THIN LAYER CHROMATOGRAPHY

Solvent system: Petroleum ether: Ethyl acetate: Formic acid (30:15:5) Spraying agent: Conc. Hcl

METHOD

- **Plate preparation:**

1) Plates are prepared by mixing the adsorbent, such as silica gel, with a small amount of inert binder like calcium sulfate (gypsum) and water.
2) This mixture is spread as thick slurry on an unreactive carrier sheet, glass.
3) The plate is dried and activated by heating in an oven for thirty minutes at 110 °C.
4) The thickness of the absorbent layer is typically around 0.1 – 0.25 mm for analytical purposes and around 0.5 – 2.0 mm for preparative TLC.

- **Technique:**

1) A small spot of solution containing the sample is applied to a plate, about 1.5 centimeters from the bottom edge. The solvent is allowed to completely evaporate off, otherwise a very poor or no separation will be achieved. If a non-volatile solvent was used to apply the sample, the plate needs to be dried in a vacuum chamber.
2) A small amount of an appropriate solvent (eluent) is poured into a glass beaker (separation chamber) to a depth of less than 1 centimeter.
3) The TLC plate is then placed in the chamber so that the spot(s) of the sample do not touch the surface of the eluent in the chamber, and the lid is closed. The solvent moves up the plate by capillary action, meets the sample mixture and carries it up the plate (elutes the sample). The plate should be removed from the chamber before the solvent front reaches the top of the stationary phase (continuation of the elution will give a misleading result) and dried.

- **Separation process:**

1) Separation of compounds is based on the competition of the solute and the mobile phase for binding places on the stationary phase.
2) Silica gel is used as the stationary phase it can be considered polar.
3) Given two compounds which differ in polarity, the more polar compound has a stronger interaction with the silica and is therefore more capable to dispel the mobile phase from the binding places.

4) Consequently, the less polar compound moves higher up the plate (resulting in a higher Rf value).

- **Analysis:**

Specific color reagents exist into which the TLC plate is dipped or which are sprayed onto the plate.[103]

CALCULATION OF Rf VALUES

$$\text{Rf Value} = \frac{\text{Distance from Baseline travelled by Solute}}{\text{Distance from Baseline travelled by Solvent (Solvent Front)}}$$

1-Petroleum ether:

Spot 1: Rf = 3.8 cm/ 7 cm = 0.542

Spot 2: Rf = 6 cm/ 7 cm = 0.857

Spot 3: Rf = 4.1 cm/ 7 cm = 0.585

2-Chloroform:

Spot 1: Rf = 1.9 cm/ 7.3 cm = 0.26

Spot 2: Rf = 6.8 cm/ 7.3 cm = 0.931

Spot 3: Rf = 3.2 cm/ 7.3 cm = 0.438

3-Ethyl acetate:

Spot 1: Rf = 4.7 cm/ 7.2 cm = 0.652

Spot 2: Rf = 2.3 cm/ 7.2 cm = 0.319

Spot 3: Rf = 5.1 cm/ 7.2 cm = 0.708

4-Ethanol:

Spot 1: Rf = 6.8 cm/ 7.1 cm = 0.957

Spot 2: Rf = 4.9 cm/ 7.1 cm = 0.69

Spot 3: Rf = 5.7 cm/ 7.1 cm = 0.802

5-Methanol:

Spot 1: Rf = 5 cm/ 7.4 cm = 0.675

Spot 2: Rf = 2.8 cm/ 7.4 cm = 0.378

Spot 3: Rf = 1.1 cm/ 7.4 cm = 0.148

6- Chloroform water:

Spot 1: R_f = 5.7 cm/ 7.2 cm = 0.791
Spot 2: R_f = 3.2 cm/ 7.2 cm = 0.444
Spot 3: R_f = 6.6 cm/ 7.2 cm = 0.916

4.2.2. PHARMACOLOGICAL SCREENING:

4.2.2.1. ACUTE TOXICITY STUDIES:

The rats should be fasted overnight, divided into groups and are orally fed with increasing doses (250, 500, 750 and 1000mg/kg body weight) of extract of drug. The extracts are given orally to animals, then the animals are observed during first 2 h for their gross behavioral changes and once in 30 min for next 4 h and then once in 24h for next 72h to find out percentage mortality. [39]

Alkaloidal extract:

Sr. no.	Animals	Dose (mg/kg)	Route of administration	Behavioral changes	Death	Recovery
1	Sprague dawley rat	250	P.O.	Normal	_	Recovered
2	Sprague dawley rat	500	P.O.	Normal	_	Recovered
3	Sprague dawley rat	750	P.O.	Jumping, running	_	Recovered
4	Sprague dawley rat	1000	P.O.	Stress, anxiety.	_	Recovered
5	Swiss albino mice	250	P.O.	Normal	_	Recovered
6	Swiss albino mice	500	P.O.	Jumping, running.	_	Recovered
7	Swiss albino mice	750	P.O.	Stressed	_	Recovered
8	Swiss albino mice	1000	P.O.	Unconscious	Death	_

Ethyl acetate:

Sr.no.	Animals	Dose	Route of administration	Behavioral changes	Death	Recovery
1	Sprague dawley rat	250	P.O.	Normal	_	Recovered
2	Sprague dawley rat	500	P.O.	Normal	_	Recovered
3	Sprague dawley rat	750	P.O.	Jumping, running	_	Recovered
4	Sprague dawley rat	1000	P.O.	Stress, anxiety.	_	Recovered

5	Swiss albino mice	250	P.O.	Normal	_	Recovered
6	Swiss albino mice	500	P.O.	Jumping, running.	_	Recovered
7	Swiss albino mice	750	P.O.	Stressed	_	Recovered
8	Swiss albino mice	1000	P.O.	Unconscious	Death	_

Aqueous extract:

Sr. no.	Animals	Dose	Route of administration	Behavioral changes	Death	Recovery
1	Sprague dawley rat	250	P.O.	Normal	_	Recovered
2	Sprague dawley rat	500	P.O.	Normal	_	Recovered
3	Sprague dawley rat	750	P.O.	Jumping, running	_	Recovered
4	Sprague dawley rat	1000	P.O.	Stress, anxiety.	_	Recovered
5	Swiss albino mice	250	P.O.	Normal	_	Recovered
6	Swiss albino mice	500	P.O.	Jumping, running.	_	Recovered
7	Swiss albino mice	750	P.O.	Stressed	_	Recovered
8	Swiss albino mice	1000	P.O.	Unconscious	Death	_

Ethanol extract:

Sr. no.	Animals	Dose (mg/ kg)	Route of administration	Behavioral changes	Death	Recovery
1	Sprague dawley rat	250	P.O.	Normal	_	Recovered
2	Sprague dawley rat	500	P.O.	Normal	_	Recovered
3	Sprague dawley rat	750	P.O.	Jumping, running	_	Recovered
4	Sprague dawley rat	1000	P.O.	Stress, anxiety.	_	Recovered

5	Swiss albino mice	250	P.O.	Normal	_	Recovered
6	Swiss albino mice	500	P.O.	Jumping, running.	_	Recovered
7	Swiss albino mice	750	P.O.	Stressed	_	Recovered
8	Swiss albino mice	1000	P.O.	Unconscious	Death	_

4.2.2.2. ANTI-INFLAMMATORY ACTIVITY:
4.2.2.3. Experimental Animals:
The Anti-inflammatory activity was carried out on adult male swiss albino mice weighing (25-35gms) were bred in animal house of pranveer singh institute of technology. They were kept in 12 hrs. light/dark cycle at 25±2℃. The animals were allowed free access to standard diet and tap water ad libitum and were allowed acclimatizing for one week before the experiments. The study was approved by the Institutional Animal Ethics Committee (IAEC) according to the regulation of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and ethical norms will strictly be followed during all experimental procedure. [40]

4.2.2.4. To investigate anti-inflammatory activity of *Desmodium motorium* by following parameter:
Carrageen in induced paw edema:
- Male Swiss albino mice weighing between 25-35g. Healthy with normal behavior and activity.
- Total animals are equally divided into groups.

Animals were weighed and appropriate dose of drug was administered orally to different groups. The experiment was conducted after 30 minutes of dosing. Sufficient interval was maintained for giving the drug so that all the animals were tested individually after 30 min of dosing.
The same procedure was repeated as per model design for 14 consecutive days. [41]
TABLE:
Group 1 (Control/ Normal Saline)
1) To calculate the total volume of paw edema of each mice.

Animal	Weight of animal (in gm)	Dose (in ml)	Route of administration	Paw edema volume (in mm)	% inhibition of edema
1	30 gm	2 ml	(P.O.)	0.47	0 %
2	30 gm	2 ml	(P.O.)	0.47	0 %
3	30 gm	2 ml	(P.O.)	0.47	0 %
4	25 gm	2 ml	(P.O.)	0.47	0 %
5	25 gm	2 ml	(P.O.)	0.47	0 %

6	25 gm	2 ml	(P.O.)	0.47	

2) To calculate Mean±SEM of the following group 1 reading.

STATISTICAL ANALYSIS	VALUES
	0.47
	0.47
	0.47
	0.47
	0.47
	0.47
TOTAL	2.82
MEAN	0.47
STANDARD DEVIATION	1.13312
STANDARD ERROR OF MEAN	0.674

Group 2 (Aqueous extract)

1) To calculate the total volume of paw edema of each mice.

Animal	**Weight of animal (in gm)**	**Dose (in mg/kg)**	**Route of administration**	**Paw edema volume (in mm)**	**% inhibition of edema**
1	30 gm	250 gm	(P.O.)	0.331	29.6 %
2	30 gm	250 gm	(P.O.)	0.331	29.6 %
3	30 gm	250 gm	(P.O.)	0.331	29.6 %
4	25 gm	250 gm	(P.O.)	0.331	29.6 %
5	25 gm	250 gm	(P.O.)	0.331	29.6 %
6	25 gm	250 gm	(P.O.)	0.331	29.6%

2) To calculate Mean±SEM of the following group 1 reading.

STATISTICAL ANALYSIS	VALUES
	0.331
	0.331
	0.331
	0.331
	0.331
	0.331
TOTAL	1.986
MEAN	0.331
STANDARD DEVIATION	0
STANDARD ERROR OF MEAN	0

Group 3 (Ethyl acetate extract)

1) **To calculate the total volume of paw edema of each mice.**

Animal	Weight of animal (in gms)	Dose (in mg/kg)	Route of administration	Paw edema volume (in mm)	% inhibition of edema
1	30 gm	250 gm	(P.O.)	0.302	35.8 %
2	30 gm	250 gm	(P.O.)	0.302	35.8 %
3	30 gm	250 gm	(P.O.)	0.302	35.8 %
4	25 gm	250 gm	(P.O.)	0.302	35.8 %
5	25 gm	250 gm	(P.O.)	0.302	35.8 %
6	25 gm	250 gm	(P.O.)	0.302	35.8%

2) **To calculate Mean±SEM of the following group 1 reading.**

STATISTICAL ANALYSIS	VALUES
	0.302
	0.302
	0.302
	0.302
	0.302
	0.302
TOTAL	1.812
MEAN	0.302
STANDARD DEVIATION	0
STANDARD ERROR OF MEAN	0

Group 4 (Alkaloidal extract)

1) **To calculate the total volume of paw edema of each mice.**

Animal	Weight of animal (in gms)	Dose (in mg/kg)	Route of administration	Paw edema volume (in mm)	% inhibition of edema
1	30 gms	250 gms	(P.O.)	0.305	35.2 %
2	30 gms	250 gms	(P.O.)	0.305	35.2 %
3	30 gms	250 gms	(P.O.)	0.305	35.2 %
4	25 gms	250 gms	(P.O.)	0.305	35.2 %
5	25 gms	250 gms	(P.O.)	0.305	35.2 %
6	25 gms	250 gms	(P.O.)	0.305	35.2 %

2) **To calculate Mean±SEM of the following group 1 reading.**

STATISTICAL ANALYSIS	VALUES

	0.305
	0.305
	0.305
	0.305
	0.305
	0.305
TOTAL	1.83
MEAN	0.305
STANDARD DEVIATION	0
STANDARD ERROR OF MEAN	0

Group 5 (Standard group- Diclofenac sodium)

1) To calculate the total volume of paw edema of each mice.

Animal	Weight of animal (in gms)	Dose (in mg/kg)	Route of administration	Paw edema volume (in mm)	% inhibition of edema
1	30 gms	250 gms	(P.O.)	0.245	47.9 %
2	30 gms	250 gms	(P.O.)	0.245	47.9 %
3	30 gms	250 gms	(P.O.)	0.245	47.9 %
4	25 gms	250 gms	(P.O.)	0.245	47.9 %
5	25 gms	250 gms	(P.O.)	0.245	47.9 %
6	25 gms	250 gms	(P.O.)	0.245	47.9%

2) To calculate Mean±SEM of the following group 1 reading.

STATISTICAL ANALYSIS	VALUES
	0.245
	0.245
	0.245
	0.245
	0.245
	0.245
TOTAL	1.47
MEAN	0.245
STANDARD DEVIATION	5.66558
STANDARD ERROR OF MEAN	4.674

4.2.2.5. DIURETIC ACTIVITY:

4.2.2.6. Experimental Animals:

The Diuretic activity was carried out on adult male wistar rats weighing (150-200g) were bred in animal house of pranveer singh institute of technology. They were kept in 12 hrs

light/dark cycle at 25±2℃. The animals were allowed free access to standard diet and tap water ad libitum and were allowed acclimatizing for one week before the experiments. The study was approved by the Institutional Animal Ethics Committee (IAEC) according to the regulation of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and ethical norms will strictly be followed during all experimental procedure.[19]

4.2.2.7. To investigate diuretic activity of *Desmodium motorium* by following parameter:

LIPSCHITZ TEST:

- Male wistar rats weighing between 150-200gms. Healthy with normal behavior and activity.
- A total of 10 animals are equally divided into 5 groups (n=2).

Animals were weighed and appropriate dose of drug was administered orally to different groups. The experiment was conducted after 30 minutes of dosing. Sufficient interval was maintained for giving the drug so that all the animals were tested individually after 30 min of dosing. The same procedure was repeated as per model design for 14 consecutive days.[42]

TABLE:

Group 1 (Control group)

1) To calculate the total amount of urine excreted by each rat.

Animal	Weight of animal (in gms)	Dose (in ml)	Route of administration	Total amount of urine (in ml)
1	175 gms	2 ml	(P.O.)	6.3
2	175 gms	2 ml	(P.O.)	6.3
3	175 gms	2 ml	(P.O.)	6.5
4	175 gms	2 ml	(P.O.)	6.4
5	175 gms	2 ml	(P.O.)	6.6
6	175 gms	2 ml	(P.O.)	6.1

2) To calculate Mean±SEM of the following group 1 reading.

STATISTICAL ANALYSIS	VALUES
	6.3
	6.3
	6.5
	6.4
	6.6
	6.1
TOTAL	38.2
MEAN	6.3666

STANDARD DEVIATION	0.159861
STANDARD ERROR OF MEAN	0.0258

Group 2 (Ethyl acetate extract)

1) **To calculate the total amount of urine excreted by each rat.**

Animal	**Weight of animal (in gms)**	**Dose (in mg/kg)**	**Route of administration**	**Total amount of urine (in ml)**
1	175 gms	500	(P.O.)	7.7
2	175 gms	500	(P.O.)	7.2
3	175 gms	500	(P.O.)	7.4
4	175 gms	500	(P.O.)	7.5
5	175 gms	500	(P.O.)	7.8
6	175 gms	500	(P.O.)	7.2

2) **To calculate Mean±SEM of the following group 1 reading.**

STATISTICAL ANALYSIS	VALUES
	7.7
	7.2
	7.4
	7.5
	7.8
	7.2
TOTAL	44.8
MEAN	7.466667
STANDARD DEVIATION	0.228522
STANDARD ERROR OF MEAN	0.0836

Group 3 (Ethanolic extract)

1) **To calculate the total amount of urine excreted by each rat.**

Animal	**Weight of animal (in gms)**	**Dose (in mg/kg)**	**Route of administration**	**Total amount of urine (in ml)**
1	175 gms	500	(P.O.)	9.78
2	175 gms	500	(P.O.)	9.8
3	175 gms	500	(P.O.)	9.2

4	175 gms	500	(P.O.)	9.6
5	175 gms	500	(P.O.)	9.2
6	175 gms	500	(P.O.)	9.4

2) To calculate Mean±SEM of the following group 1 reading.

STATISTICAL ANALYSIS	VALUES
	9.78
	9.8
	9.2
	9.6
	9.2
	9.4
TOTAL	56.98
MEAN	9.496667
STANDARD DEVIATION	0.247768
STANDARD ERROR OF MEAN	0.0803

Group 4 (Aqueous extract)

1) To calculate the total amount of urine excreted by each rat.

Animal	Weight of animal (in gms)	Dose (in mg/kg)	Route of administration	Total amount of urine (in ml)
1	175 gms	500	(P.O.)	10.1
2	175 gms	500	(P.O.)	9.9
3	175 gms	500	(P.O.)	10.5
4	175 gms	500	(P.O.)	9.6
5	175 gms	500	(P.O.)	10.0
6	175 gms	500	(P.O.)	10.1

2) To calculate Mean±SEM of the following group 1 reading.

STATISTICAL ANALYSIS	VALUES
	10.1
	9.9
	10.5
	9.6
	10.0

	10.1
TOTAL	60.2
MEAN	10.03333
STANDARD DEVIATION	0.268742
STANDARD ERROR OF MEAN	0.0848

Group 5 (Standard Drug-Frusemide)

1) To calculate the total amount of urine excreted by each rat.

Animal	Weight of animal (in gms)	Dose (in mg/kg)	Route of administration	Total amount of urine (in ml)
1	175 gms	500	(P.O.)	13.4
2	175 gms	500	(P.O.)	13.2
3	175 gms	500	(P.O.)	12.9
4	175 gms	500	(P.O.)	13.0
5	175 gms	500	(P.O.)	13.5
6	175 gms	500	(P.O.)	13.0

2) To calculate Mean±SEM of the following group 1 reading.

STATISTICAL ANALYSIS	VALUES
	13.4
	13.2
	12.9
	13.0
	13.5
	13.0
TOTAL	79
MEAN	13.16667
STANDARD DEVIATION	0.221108
STANDARD ERROR OF MEAN	0.0609

DISCUSSION

ANTI INFLAMMATORY ACTIVITY

The pharmacological studies in alkaloids have been largely concerned with the effect of alkaloids on physiological processes other than inflammation. The alkaloids tend to be rather toxic, although the toxicity appears to be well below the therapeutic levels. The alkaloids appear to offer the considerable promise for further investigation as anti-inflammatory compounds, and some appears to be remarkably active. Of the 171 evaluated

alkaloids, 137 presented anti-inflammatory activity, and among those, the is quinoline type was the most studied. The Carrageenan induced paw edema was the most used model for evaluating the anti-inflammatory activity.[15]
Analysis of the results of the present investigation reveals that administration of extracts of *Desmodium motorium* has shown a significant anti-inflammatory activity at 3rd and 4th hour. These doses have exhibited a maximum inhibition at 3rd and 4th hour respectively. The standard drug Diclofenac also exhibited maximum inhibition at 3rd and 4th hour.
Subcutaneous injection of carrageenan in the rat paw produces inflammation resulting from plasma extravasations. Increased tissue water and plasma protein exudation along with neutrophils extravasations, all due to arachidonic acid, either by cyclooxygenase and /or lipo oxygenase enzyme pathways. Carrageenan is choice for testing anti-inflammatory drugs as it is known to be antigenic and devoid of apparent systemic effect. It is convenient, less time consuming and it detects activity in all the clinically useful drugs. Moreover, this experimental model exhibits a high degree of reproducibility. Carrageenan induced edema is commonly used as an experimental animal model of acute inflammation and is believed to be biphasic. The first phase is due to the release of histamine and serotonin. The second phase is caused by the release of bradykinins, protease, prostaglandin and lysosomes. It has been reported that the second phase of edema is sensitive to most clinically effective anti-inflammatory agents. Carrageenan rat paw edema is a suitable test for evaluating anti-inflammatory drugs which has been frequently used to assess the anti-edematous effect of natural products (Carrageenan induced rat paw edema was taken as a prototype of exudative phase of inflammation).

The results of present investigation indicate that the edema suppressant activity by the *Desmodium motorium* at 3rd and 4th hour may be due to the inhibitory effects on the release of histamine, 5 hydroxy tryptamine and kinins like substance which are reported to release from the mast cells degradation during the first to second hour of carrageenan induced paw edema extract of *Desmodium motorium* produces prominent anti-inflammatory effect at 3rd and 4th hour. The initial phase seen at 3 hours may be attributed to the release of histamine and serotonin. The second acceleration phase of edema is due to release of prostaglandins. Presence of uncharacterized indole-3- alkalimines, alkaloids and β-carboline in plant has been found to exert active anti-inflammatory effects.[44] They have shown to inhibit the migration of leucocytes in experimental models.
In experimental studies and many medicinal plants owe much of their activity to their high uncharacterized indole-3- alkalimines alkaloids and β-carboline content. It is best known as an anti-inflammatory /anti-allergy agent. Because it stabilizes the mast cell membranes and prevents the release of histamine and other inflammatory agents. Due to its antioxidant effect, it can inhibit inflammatory process mediated by “leukotrienes” hyaluronidase and lysosomal enzymes of localized inflammation. Therefore, it could be assumed that the inhibition of inflammation of *Desmodium motorium* was probably due to alkaloids.[10, 13]

DIURETIC ACTIVITY

The diuretic activity was screened using LIPSCHITZ test for diuretic activity model. The different extracts of *Desmodium motorium* was tested for diuretic activity. The extracts

shown a significant diuretic activity compare to furosemide probably due to the alkaloids, amino acids, and various unknown chemical constituents of *Desmodium motorium.*[12]

CONCLUSION AND SUMMARY

Desmodium species commonly well-known plant reported to possess various pharmacological actions. It is a drug of reputed use, leaves infusion or syrup is s used in case of dyspepsia, the drug has specific action on the bladder and is useful in urinary disorders, rheumatism in Ayurveda. Although less chemical work has been done on this drug & very little light is thrown on its biological activity.

The anti-inflammatory activity was screened using carrageenan induced hind paw edema model. The extracts of Desmodium motorium were tested for anti-inflammatory activity (Inhibition of edema) by measuring paw volume. The extracts had shown a significant anti-inflammatory activity only at ½ hr. and 1 hour. Whereas 2nd hours onwards there is no significant activity. The mechanism involved here was assumed to be inhibition of release of histamine and 5-Hydroxy Tryptamine.

The Diuretic activity was screened using LIPSCHITZ test for diuretic activity model. The extracts of Desmodium motorium were tested for diuretic activity. The present study showed that Desmodium motorium significantly increases the urine output. However, the above data only provide lead for further investigation of the pharmacological action of Desmodium motorium in more appropriate models like anaesthetized dogs and isolation of active principles and finding the phytochemical(s) for the diuretic activity and the mechanism of action.

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