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Evaluation of Analgesic Activity of Ethanol Extract of Gyrocarpus Americanus Jacq

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Abstract**—***Gyrocarpus americanus is a plant species that has been traditionally used in folk medicine for its medicinal properties. The plant is found in various regions of Africa and India. Its extracts have been investigated for their potential analgesic activity, and studies have suggested that ethanol extracts of Gyrocarpus americanus wild may exhibit analgesic effects. One study found that an ethanol extract of Gyrocarpus americanus leaves exhibited dose-dependent analgesic activity in mice, while another study found that an ethanol extract of the plant's bark exhibited significant analgesic activity in rats. The analgesic effects may be attributed to the presence of alkaloids or may be mediated by the opioid system. However, further research is needed to fully understand the mechanisms of action and to determine the safety and efficacy of these extracts for human use.*

Keywords**—***Gyrocarpus americanus, Phytochemical, Analgesic Activity, Ethanol extract.*

I. INTRODUCTION

Gyrocarpus americanus, commonly known as gyrate milkwood or kahikatea, is a plant species that belongs to the family Hernandiaceae. It is a tree that is native to various regions of Africa and India and has been traditionally used in folk medicine for its medicinal properties. The plant's extracts have been investigated for their potential therapeutic applications, including analgesic activity.

Pain is a common and distressing symptom that can arise from various conditions and can greatly affect a person's quality of life. Analgesics are drugs that relieve pain, and they are one of the most commonly used medications in clinical practice. However, some analgesics can have side effects and may be addictive, leading to a search for alternative natural sources of pain relief.

The use of natural products as potential analgesics has gained interest due to their perceived safety and efficacy. Gyrocarpus americanus has been identified as a plant species that may have analgesic activity, and studies have investigated its potential use in pain management. This introduction

sets the stage for exploring the potential analgesic properties of Gyrocarpus americanus and the studies conducted to investigate its medicinal properties.

II. AIM & OBJECTIVES

- The use of these drugs as analgesic agents has not always been successful due to adverse side effects, such as gastric lesions caused by NSAIDs, and tolerance and dependency caused by opiates. In the meantime, NSAIDs and opiates are being replaced by analgesic drugs without those effects all over the world. According to WHO, about 80% of the world's population still use mostly plant-based medicines and attention has been paid during this process to the efficacy of these drugs due to their affordability and low side effects.
- *Gyrocarpus americanus* Jacq was studied for its analgesic activity. A pain model was employed on mice.
- *Gyrocarpus americanus* Jacq is a plant from North America. Some sources claim it has medicinal properties such as anti-bacterial, anti-oxidant, anticancer, and analgesic properties without any scientific verification.
- Many species within the same genus may contain active compounds of the same nature, and therefore, contain similar bioactive compounds. The fact that *Gyrocarpus americanus* Jacq provides pain relief led to its scientific validation.
- A review of the literature shows that *Gyrocarpus americanus* Jacq possesses an untapped potential in phytochemistry, toxicology, and analgesic effects.
- An experimental study of the phytochemical constituents of *Gyrocarpus americanus* Jacq is being conducted.

III. PLANT PROFILES

Gyrocarpus americanus Jacq.

3.1 Plant Introduction

Botanical name :*Gyrocarpus americanus* Jacq.

Family :Gyrocarpaceae or Hernandiaceae

Common Name : Helicopter Tree

Synonyms :Gyrocarpus asiaticus Willd.

Vernacular Names : Hindi - Zaitun

Tamil - Chaivavatala, Tanakku

English - Stinkwood

Telugu - Tanuku, Nallaponaku

Kannada - Kadubende, Pollika

Figure 1: - Leaves of *Gyrocarpus americanus* Jacq

A. *3.4 DISTRIBUTION*

Gyrocarpus americanus Jacq. Trees of this genus can reach a height of 20 meters, sometimes even less. Bark: Pale grey-white bark at first, becoming rough and dark later Twigs brittle. The alternate, rounded leaves are rounded with 3 lobes, on thin leaves stalks to 8 cm. Three yellow veins separate them from the base of the leaf. Dark above and gray-green below, turning yellow before leaf fall. Young trees have broad, deeply 3-lobed leaves that are crowded near the ends of the branches, while older trees have smaller and nearly entire blades. There are many male flowers with some bisexual and female flowers. The flowers are yellow-green, a foul odor, and borne in dense clusters on the trees. Hard, woody, oval fruit with two thin wings, 5-8 cm long and well-marked with veins. It breaks off from the tree and disperses into the air by the wind as the wings rotate as if they were helicopters[6, 7, 8].

B. *PHYTOCHEMISTRY*

Phytochemical analysis of Gyrocarpus americanus has revealed the presence of several bioactive compounds. These include alkaloids, flavonoids, tannins, and phenolic compounds. These phytochemicals are believed to contribute to the plant's therapeutic properties and may have potential pharmacological activities [7, 9].

It is important to note that the phytochemical composition of Gyrocarpus americanus can vary depending on factors such as geographic location, plant part, and extraction methods [8, 11]. Further scientific research is needed to fully characterize the phytochemical profile and identify the specific constituents present in Gyrocarpus americanus. Such studies will contribute to a better understanding of its potential pharmacological activities and therapeutic applications.

IV. PHARMACOGNOSTIC STUDY OFGYROCARPUS AMERICANUS LEAVES

A. *Gyrocarpus americanus* Jacq. Leaves Phytochemical Investigation

✓ **Reagents, solvents, and chemicals**

Ethanol and methanol are solvents

- **Reagents.** There are a number of reagents that are used for this and these include Mayer's reagent, Dragendorf's reagent, Wagner's reagent, Hager's reagent, Ninhydrin reagent, Carr-Price reagent, Libermann-buchard's reagent, and Fehling's solution.
- **Chemicals.** In a test tube, add 10% Potassium dichromate solution, 10% Aqueous lead acetate solution, 10% Hydrochloric acid, Sodium carbonate, 0.1 N sodium hydroxide, acetic anhydride, 10% Potassium dichromate solution.

*SD Fine chemicals provides all the chemicals and solvents for analytical grade.

✓ **Extraction process**

A coarse powder was produced from (Family: Gyrocarpaceae). In a soxhlet apparatus, the powder was stuffed with ethanol (60 - 80℃), then extracted with 95% methanol. By distilling under reduced pressure, the solvent is removed from the sample and preserved in desiccators for future experiments [14, 15].

B. **Preliminary studies in phytochemistry**

✓ **Chemical testing**

- In this study, ethanol and methanol extracts of *Gyrocarpus americanus* Jacq leaves were examined for their phytochemical properties. It belongs to the Gyrocarpeceae family.

✓ **Alkaloids are detected**

- Several drops of dilute hydrochloric acid were added to ether and methanol extracts, and the mixtures were stirred separately. Various alkaloid reagents were used to test the filtrate.
- **Mayer's test**, which involves treating an extract with potassium mercuric iodide (Mayer's reagent) and looking for cream-coloured precipitate, which indicates alkaloids are present [17, 19].
- **Dragendroff's reagent**: the test extract was treated with potassium bismuth iodide (Dragendroff's reagent) to determine whether any alkaloids were present [17, 19, 20].
- **Wagner's test:** This is a simple procedure in which a sample is treated with potassium iodide solution (Wagner's reagent) and a brown precipitate is formed to indicate the presence of alkaloids [19, 20].
- **Testing with Hager's reagent**: a saturate of picric acid (Hager's reagent) formed a yellow precipitate that indicated alkaloids were present in the test extract [17, 19, 20].

✓ **Testing solution preparation**

An alcohol or hydro-alcoholic solution was used to dissolve the test extract.

✓ **Cardiac Glycoside Test**

- ***Testing by Baljet.*** A sodium picrate treatment was applied to the test extract [22]. This induced the formation of yellow to orange pigments.

- ***Testing Raymond's theory.*** A hot methanol alkali solution of dinitrobenzene was used to treat the test extract [23, 28]. In the presence of cardiac glycosides, violet colour formation was observed.

We tested the water for bromine. Bromine water was used to dissolve the test extract. Presence of cardiac glycosides was detected in the yellow precipitate that formed.

- ***Digitaloxose is diagnosed using the Keller-Killiani test. Two layers were formed after mixing the sample extract with FeCl3 solution and adding H_2SO_4 that contained $FeCl_3$ solution*** [21,24]**.** In the upper layer, cardiac glycosides appear as bluish green.
- ***Test of legality.*** Adding sodium nitroprusside solution to the test extract adds pink to red color to the extract, indicating cardiac glycosides [22, 23].

✓ **Anthraquinone glycosides are tested**

- ***Birthtrager's test.*** After boiling for five minutes in 10% sulphuric acid, the powdered drug was removed from the solution. Benzene was added in equal measure to the filtrate after it was filtered and cooled. Separated benzene layer was treated with 10% ammonia solution half as much as its volume. The presence of anthraquinones can be determined by the rose-pink color of the ammonical layer [22, 25].
- ***Test adapted from Borntrager. A more severe hydrolysis environment is required for C-glycosides of anthraquinones.*** A 5% solution of FeCl3 and diluted HCl were used to hydrolyze the drug. We followed the rest of Borntrager's test procedure for hydrolyzed extract [22, 25, 28].

C. CYANOGLYCOSIDES

- ***Testing Grignard.*** In a test tube containing a small amount of powdered drug in water, strips of sodium picrate filter paper were inserted between split cork stoppers. To avoid contact with the inner surface of the tube, the paper was not touched. Half an hour later, the contents were warmed. Cyanogenetic glycosides have a red color on the strips [25, 27, 30].

✓ **Glucosamine Glycosides**

Fluorescent properties were observed in alcohol extracts made alkaline.

✓ **Carbohydrates are detected**

We dissolved methanol and ethanol extracts separately in distilled water, and then filtered the mixture. We tested the filtrate for different carbohydrates using different tests [25, 28].

- **Describe Molisch's test in your own words. A solution of a-Naphthol and a few drops of condensation were applied to filtrates to treat them.** The test tube was then filled

with sulphuric acid. Carbohydrates are found at the junction of the two liquids because they form a violet ring [28, 29].

- **The test of Fehling.** In addition to the dilution of hydrochloric acid, the filtrates were heated on a water bath for 30 minutes. Sodium hydroxide was then used to neutralize the solutions. A few minutes later, the solutions were heated on a water bath with equal quantities of Fehling's A and B. Red-orange precipitates form when reducing sugars are present [29, 31].
- **The test of Benedict.** Benedict's reagent was added to the filtrates and the water bath was heated for a few minutes. It is thought that reducing sugars would form a red-orange precipitate [28, 29, 31].

We also hydrolyzed a small portion of extract with diluted hydrochloric acid in a water bath for a few hours to determine glycoside content.

(a) Phytosterols can be detected

Hydroxide solution of alcoholic potassium was reacted separately with ethanol and methanol extracts until complete saponification occurred. Using distilled water and solvent ether, the diluted mixtures were extracted. Liebermann-Burchard's test was performed on the residue of ethereal extract that was evaporated to dryness [29, 30].

✓ **Experimenting with Liebermann-Burchard**

In the test tube, ether residues were treated with a few drops of acetic anhydride dissolved in boiling water. After cooling, 1 ml of sulphuric acid was added. In the presence of steroid and triterpenoid compounds, a brown ring forms at the intersection of two liquids, and the upper layer is green [29, 31, 33].

(b) Triterpenoids

❖ ***Test extract preparation***

Extract was dissolved in ethanol and methanol to prepare the test extract solution.

- ***This is the Salkow test.*** The test solution was added with a few drops of concentrated sulphuric acid, shaken, and when left to stand the lower layer turns golden yellow, indicating triterpenes [30, 31].

- ***Test Burchard-Libermann.*** I added acetic anhydride to the extract test solution after mixing it well. A reddish color is produced in the lower layer of the tube when 1 ml of concentrated sulphuric acid is added from the sides [30, 31].

(c) Fat and oil detection (c)

- **Test on the spot.** Two filter papers were used to press separate samples of methanol and ethanol extracts. Filter paper stained with oil indicates that there is a fixed oil present [31, 33].
- **Test of sacrifice.** The ethanolor methanol extract was treated with 0.5 N alcoholic potassium hydroxide in addition to a drop of phenolphthalein. A water bath was used to heat the mixture for 1 to 2 hours. If soap forms or alkali partially neutralizes, fixed oil or fat is present [31, 33].

(d) Saponins are detected

- **Test using foam.** A 20 ml sample of alcohol extract was diluted to about 1 ml with distilled water, then shaken for 15 minutes in a graduated cylinder[38, 40, 41]..

  Presence of saponins is indicated by the formation of froth above the surface.

✓ **The detection of phenolic components and tannins**

The presence of phenolic compounds and tannins was tested in small quantities of aqueous and alcohol extracts diluted separately in water [31, 33, 35].

- **Chloride test using ferric chloride.** We added some ferric chloride solution (5% in water) to the test solutions. When phenolic compounds or tannins are present, a bluish-black or greenish-black color is formed.
- **Test with gelatin.** A few drops of 10% sodium chloride solution containing 1% gelatin were added to the test solutions. Presence of tannins is indicated by a white precipitate.
- **Analyzing lead acetate.** Few drops of the lead acetate solution were added to the test solution. The presence of tannins led to the formation of white precipitates. The presence of flavonoids is indicated by yellow precipitates.

Testing with bromine in water. We added some drops of aqueous bromine solution to the test solution. The precipitate formed indicated the presence of tannins [31, 35].

✓ **The detection of protein and amino acid free molecules**

The presence of proteins and free amino acids in alcohol and aqueous extracts was tested by diluting the extracts in water separately and subjecting them to various tests [30, 38].

- **A biouret test.** Several drops of a solution of copper sulphate (0.7%) were added to test solutions. The presence of amino acids is indicated by a purplish violet color [30, 32].

- **A test using Ninhydrin.** Ninhydrin solution was added to the test solutions in a water bath after they had been analyzed. When bluish color forms, amino acids are present [30, 35, 37].

✓ **Gums and mucins can be detected**

In 25 ml of absolute methanol, 10 ml of extract was added continuously while stirring. Carbohydrate content of precipitate was determined.

✓ **In today's world, there are a lot of ways to detect flavonoids.**

- **Test shinada.** As part of the test, magnesium metal fragments and concentrated hydrochloric acid were added to the test solution. Magnesium metal fragments and concentrated hydrochloric acid were added to the test solution [36, 41].
- **A test using alkaline reagents.** Sodium hydroxide solution was added to some of the test solutions. An intense yellow color that diminishes when acid is added indicates flavonoids are present [36, 38, 41].

D. **Gyrocarpus americanus Jacq.Pharmacognostic Analysis of the Leaves, Macroscopical Studies**

Fresh *Gyrocarpus americanus* Jacq leaves as seen under the microscope. In table 5.1 and 5.2 we show the results from the study of (Family: Gyrocarpaceae).

Table I: A macroscopic comparison of *Gyrocarpus americanus* Jacq is presented.

Aspects	**Notes**		
	Dogs	**Leaf**	**Blooms**
Various	Pale grey-white	Pale grey-green	green-yellow
aroma	Odourless	Good smell	Disgusting
smell	Description	There is a hint of bitterness	Characteristic
Feel	Sleek	But Rugged	Sleek
appearance	turns to rough and brittle later	Plants alternating, petiolate, with broadly ovate blades	forming spheres with dense branches
Large	(20-30m)	7cm x 10cm	inyt

E. ASSESSMENT OF PHYSICOCHEMICAL PARAMETERS

***Water Content**

Hydration in *Gyrocarpus americanus* Jacq leaves as a percentage. Weight/weight 8.5 of the extracts were studied by using ethanol and methanol.

Table II: The ash content of *Gyrocarpus americanus* Jacq leaves is shown.

Values of ash	content. (%w/w)
Ashes in total	7.56
ash value in acid	0.86
soluble in water	1.26
Ascorbic acid value	5.46

Table III: *Gyrocarpus americanus* Jacq leaf extracts in Ethanol and Methanol: preliminary phytochemical analysis.

S. No.	Phytocomponents	Phytocomponents of *Gyrocarpus americanus* Jacq leaves.	
		Petrol Ether Extraction	Using Methanol
1.	Analkoids	-	+
2.	Sugars	-	-
3.	Proteoproteins	-	+
4.	Polyterpenoids	-	-
5.	Polyphenols	-	+
6.	Flavourings	-	+
7.	Tynanines	-	+
8.	Acycloglucosides	-	-
9.	Gummiase and mucin	-	-
10.	Saponoid	-	-
11.	Andro-steroid	+	+
12.	Fatty acids & oils	+	-

*Presence/Absence is indicated by "+"; absence by "-"

[a.])

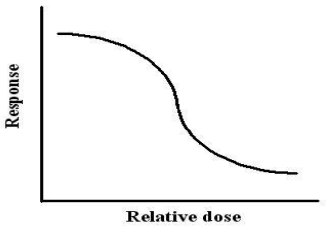
V. ACUTE ORAL TOXICITY

A. **Relationship between dose and toxicity***:*

Toxicology is the study of the relationship between dose and its effects on the exposed organism. The chief criterion regarding the toxicity of a chemical is the dose, i.e., the amount of exposure to the substance [21]. Paracelsus, who lived in the 16th century, was the first person to explain the dose-response relationship of toxic substances [21, 22].

In summary, the relationship between dose and toxicity highlights the importance of appropriate dosing to minimize the risk of adverse effects. Finding the balance between an effective dose and avoiding toxicity is a critical consideration in the development and use of pharmaceuticals and other substances.

B. **Dose-Response Model**



C. **EXPERIMENTS ON ORAL TOXICITY**

An organism is deemed toxic when it can be damaged by a substance. As well as the toxicity of an organism as a whole, toxicity can also refer to the local toxicity of a particular organ or substructural component of the organism, such as cytotoxicity of a cell or organo-toxicity of the liver [24, 26].

An acute toxicological event is usually defined as the occurrence of an adverse reaction following a single or short exposure to a substance, agent, or occurrence of an adverse reaction triggered within a short period after exposure to a substance, agent, or as an adverse reaction resulting from multiple doses given within 24 hours [26, 27].

Acute toxicity testing is done to better understand a chemical's mechanism of action as well as its biologic activity. An acute dose-finding test is often the first step in a long-term study. As a result of this test, data on acute systemic toxicity are used for hazard identification and risk management when a chemical is produced, handled, or used. Toxicological classification of chemicals currently uses LD50 values (precise and approximate) which are used by government agencies in various circumstances [26, 28, 30]. As far as 2 weeks after dosing the animals, changes in appearance and behavior will be observed day by day during the first 24 hours [28, 29]. There are a broad range of clinical signs which enable us to determine acute systemic toxicity and its progression.

D. **Principle**

Acute Toxic Class Method was used to implement the procedure. Step by step, three rats of a single sex are introduced to the acute toxic class method. The number of steps necessary to determine the acute toxicity of a substance may depend on the mortality or morbidity of the animals and the average outcomes of the tests [28, 29, 31]. Rats are used in this experiment to allow for satisfactory scientific conclusion based on available data. In accordance with the Globally Harmonized System (GHS) for the classification of chemicals with acute toxicity, the method used to define doses (2000, 300, 50, and 5mg/kg) results can be compared to rank and classify the substance. [31, 32, 35]

E. **Materials**

The extract of *Gyrocarpus americanus* Jacq in Ethanol Sodium Carboxymethyl Cellulose (SCMC) 1% w/v in the vehicle; oral needle. [33, 35, 40]

F. **Experimental Animals**

The experiment used Worster albino rats between the weights of 150 to 220 grams each. Animals were housed in polypropylene cages in a well-ventilated room with 12:12 h light and dark cycles, kept at 22.1℃ with 55.5% relative humidity during the experiment, and given a balanced rodent pellet diet and tap water ad libitum.[29, 33, 34]

G. **Experimental Procedure**

Gyrocarpus americanus extract in ethanol. Subjected to acute oral toxicity studies. Initially, *Gyrocarpus americanus* ethanol extract was given at a dose of 2000mg/kg, p.o. to albino worster rats. In order to determine whether most of the crude extracts possessed LD50 values larger than 2000

mg/kg, the dose [29, 30, 34].To overnight fasted rats, 0.2ml was administered per 100gm weight. Following the administration of Ethanol extract of *Gyrocarpus americanus* Jacq, food was withheld for another 3 to 4 hours. Toxicity was monitored. Observations were made regarding body weight, changes in eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous systems, motor activity, and behavioral patterns of the rats before and after administration. 164 Additional signs of toxicity and signs of toxicity observed included tremors, convulsions, saliva, diarrhea, lethargy, sleep, and coma [31, 34].

Mortality data and necropsies have been recorded. *Gyrocarpus americanus* Jacq ethanol extract was found to have acute oral toxicity. Observation of the autonomic and central nervous system, motor activity, and behavioral pattern was conducted on the treated rats for 14 days after administration of the Ethanol extract of *Gyrocarpus americanus* Jacq [30, 31]. In this study, there was no toxic effect or lethality found at the dose of 2000mg/kg as indicated by convulsions, salivation, diarrhea, lethargy, sleep and coma.

In order to study 1/5th (400mg/kg) of this maximum oral dose further, it was chosen to be studied in further detail. [37, 39, 40]

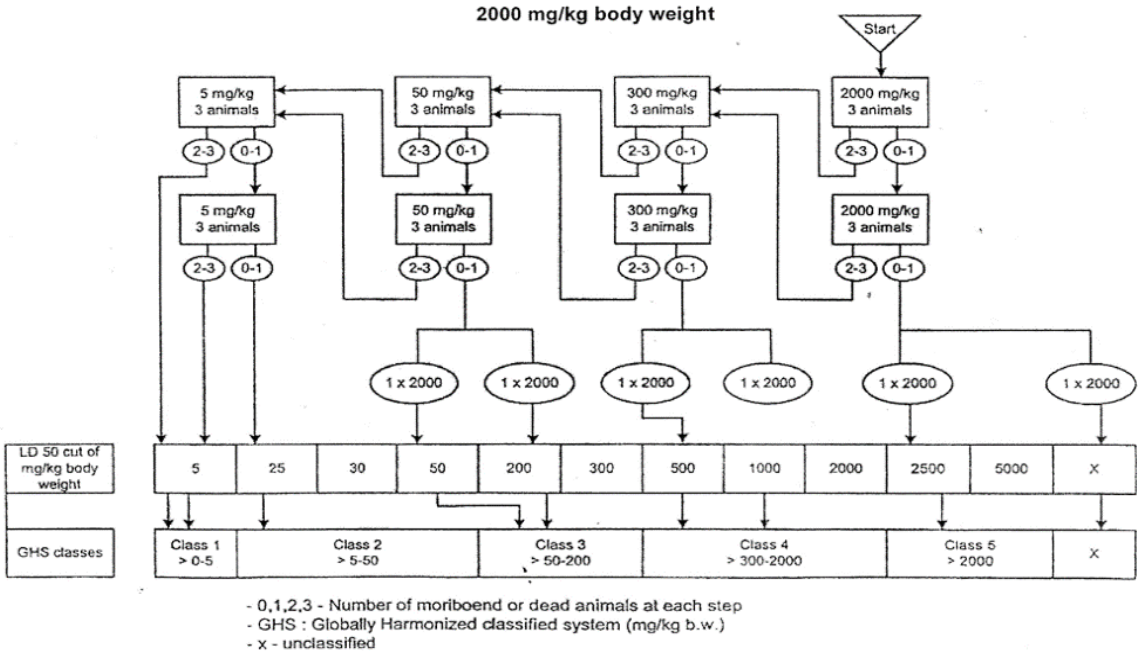


Figure 2: Flow chart for acute toxic class method starting dose

Table: IV. Acute oral toxicity of EEGA at 400mg/kg.

Maximum dosage	sex	Animals killed on dosing day (h)					Days following dosing (no animals killed)								Mortality
		1/2	1	2	3	4	1	2	3	4	5	6	7	8-14	
2 mg/kg EEGA	M/3	0	0	0	0	0	0	0	0	0	0	0	0	0	0/3
	F/3	0	0	0	0	0	0	0	0	0	0	0	0	0	0/3

Table: V. Study of the acute oral toxicity of EEGA, concluded from necropsy findings

S. No	Medications	Schedule (mg/kg)	No. of animals and sex	Average Rat Weight		Study Duration	Observations macroscopically
				prior to test	Observations after test		
1	EEGA	2000	1-Female	190gm	195gm	14 days	Found to be normal
2	EEGA	2000	1-Male	185gm	190gm	14 days	Found to be normal
3	EEGA	2000	1-Male	180gm	185gm	14 days	Found to be normal

VI. ANALGESIC ACTIVITY

H. METHODS OF EXPERIMENTATION

6.1.1 ANIMALS

Mice of either sex (25-30 g) were kept at a constant temperature of 25℃ in 12-hour light/dark cycles with free access to food and water. Further, the animals were kept in specially designed cages so that coprophagia wouldn't occur during the test. CPCSEA (Commission for the Purpose of Control and Supervision of Experiments on Animals) approved all experiments carried out based on the guidelines for the care and use of experimental animals. [38, 42, 45]

6.1.2 Studies on Acute Toxicity

G. americanus extract Ethanol toxicity, (Acute Toxic Class Method) was used to determine the acute toxicity of leaves. A dose of 2000mg/kg of the extract was not lethal to rats. To continue studies on this dose, a tenth (200mg/kg) and a fifth (400mg/kg) were chosen. [42, 45]

6.1.3 There were animals involved

A group of male albino mice (20-25g) were collected from the animal house. We kept the animals in polypropylene cages in a well-ventilated room with 12:12 hours of light and dark. During the experiment, the animals received standard pellet feed (Hindustan Lever Limited., Bangalore) and water on a daily basis. [30, 37]

6.2 PAIN CAUSED BY FORMALIN

Hunskaar and Hole performed the formalin test. We divided the mice into four groups with six each of the albino male mice weighing 20-25g. Formalin was subcutaneously injected into the dorsal hind paw of each group of animals. [40, 43, 45]

- **Design of experiments**

Group I: Received vehicle control (1% v/v SCMC, 1ml/100g)

Group II- Ethanol extract of *Gyrocarpusamericanus* (EEGA) (200mg/kg body weight p.o)

Group III- Ethanol extract of *Gyrocarpusamericanus* (EEGA) (400mg/kg body weight p.o)

Group IV- Indomethacin (10mg/kg) p.o. taken 30 minutes before formalin injection.

Mouse lick or bite time was recorded when the injected paw or leg was licked or bit. Two distinct periods of intensive licking were identified and scored separately based on the response pattern described by Tjolsen et al. The early phase (first period) was observed after the injection of formalin, and then the late phase (second phase) was observed after 20- 40

Table: VI. In the formalin test EEGA has analgesic effects

Groups	Treatment Plan	Licking		Intensity (%)
		0-5min	15-40min	
I	(1) SCMC 1% by weight, 1ml/100g (control)	53.33± 0.22	126±1.27	–
II	EEGA (200mg/kg w.w., po)	53±0.54	68.33±1.75**	45.77
III	EEGA (400mg/kg w.w.,	52 ± 0.58	47.33±0.67**	62.45

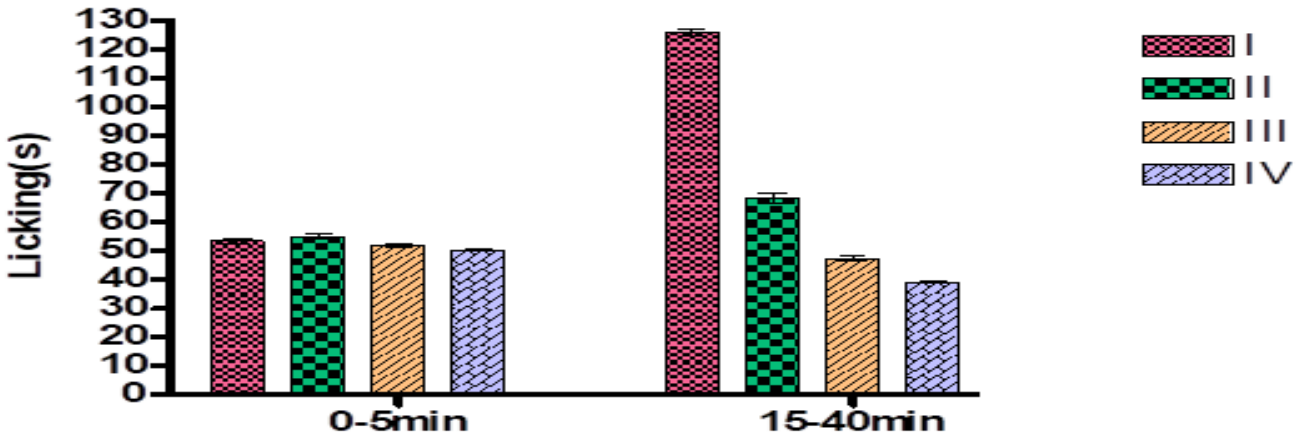
	po)			
VI	Indimetacin (10mg/kg b.w, p.o)	51.24±0.33	39.15±0.22**	68.94

***Injecting of formalin into the dorsal hind foot after 30 minutes of EEGA treatment is the next step**.

(N=6). * P0.05, ** P0.01 significant when compared with control.

Analysis of variance followed by Dunnett's test

minutes. Using the formula: (C×T)/C×100, percentage inhibition of licking is calculated by subtracting



the value of the control group from the treatment group value for each phase. [41, 44]

Figure: 3. In the formalin test, the analgesic effects of oral EEGA were compared

***Pain caused by Formalin**

The EEGA 200 mg/kg and EEGA 400 mg significantly inhibited formalin induced pain (45.77%, 62.45% in the late phase of the test. In the late phase of the study, indomethacin (10 mg/kg) (68.94) also significantly inhibited the endothelial cell proliferation (P = 0.01).

6.3 HOT PLATE METHOD

Turner describes how to use the hot plate method. Four groups of six albino mice each were divided into 20 to 25 gm males. [38, 39]

- **Design of experiments**

Group I: Received vehicle control (1% v/v SCMC, 1ml/100g)

Group II- Ethanol extract of *Gyrocarpusamericanus* (EEGA) (200mg/kg body weight p.o)

Group III - Ethanol extract of *Gyrocarpusamericanus* (EEGA) (400mg/kg body weight p.o)

Group IV- Morphine Sulfate (5mg/kg s.c) taken 30 minutes before experiment.

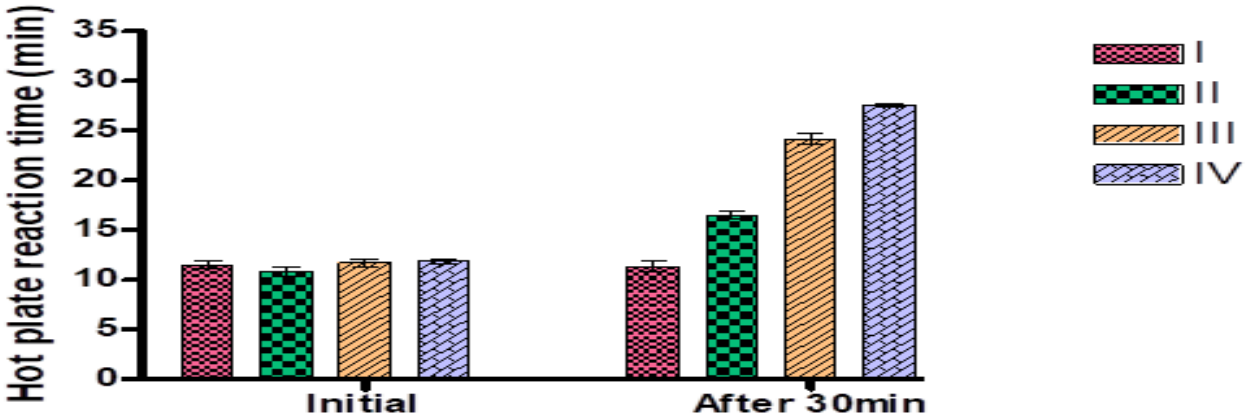
*A hot plate maintained at 55°C was used to screen the mice, which were then measured for their reaction time in seconds to jumping or licking their hind paws. 40 second cut-offs were used to avoid damage to the tissue. [34, 36, 37]

Table: 7.2. In mice, the effect of topical and oral intake of EEGA on the time required to react to a hot plate.

Group	Treatment planning	Latent Variables	
		Initial[b]	After 30 min[b]
I	One milliliter of 1% SCMC was added to 100 grams as control	11.49 ± 0.43	11.22 ± 0.45
III	EEGA (200 mg/kg body weight, PO)	10.74 ± 0.48	16.46 ± 0.44**
IV	EEGA (400 mg/kg body weight, PO)	11.54 ± 0.45	24.33 ± 0.49**
IV	Morphine Sulfate (5mg/kg s.c)	11.22 ± 0.29	27.72 ± 0.14**

*EGA treatment for 30 minutes was followed by placing the mice on a hotplate.

***A mean value and a SEM are used (N=6).**



*ANOVA followed by Dunnett's test of significance (ns - not significant, * P*0.05, and ** P*0.01).

Figure: 4. An evaluation of the effects of oral EEGA administration on the Time to Reaction on a Hot Plate Among mice

*** Reaction time to a hot plate in mice**

A significant difference in analgesia was found in the EEGA 200mg/kg and EEGA 400mg) as shown in Table 7. The maximum protective effect of morphine sulphate measured after 30min was 27.52 ± 0.133 (P = 0.01) at just 5 mg/kg, s.c. In comparison of EEGA 200mg (16.5 ± 0.4283) and (24.17 ± 0.4774) (P *0.01) 400 mg/kg of the EEGA, there was a significant protective effect.

VII. DISCUSSION

Based on the findings in the present study, ethanol extract from *Gyrocarpus americanus* has analgesic activity at both a peripheral and central level. Chemically induced nociceptive stimuli (formalin) were used to calculate its peripheral analgesic activity. 400mg of EEGA were compared with 10 mg/kg (67.33%) of indomethacin when given at 400 mg/kg (62.44%) and when given at EEGA 400 mg/kg (62.44%).

Analgesics whose actions are mediated by the central nervous system can be tested by means of the hot plate test. Normal tissues normally do not respond to NSAID's, while narcotics and local anesthetics increase the threshold for pain208. First-phase results of formalin-induced pain and hotplate tests corroborated the centrally acting protective effects of EEGA. The results of these studies indicated that mu (*) opioid receptors had the greatest impact on pharmacological actions compared to kappa (κ) and delta (δ) receptors 209-210.

The effect of morphine sulphate on the effects of combined ethanol extracts of *Gyrocarpus americanus*. A hot plate test showed that morphine sulphate solution at 5 mg/kg had very potent analgesic effect, with an active potency of 27.72 ± 0.14(P = 0.01) after thirty minutes, compared to EEGA 200 mg/kg (P = 0.05) and EEGA 400 mg/kg, (P = 0.01 - 0.02). As an opioid receptor agonist, EEGA was weaker. This indicates that the extract also acted through opioid receptors that were more centrally located rather than peripherally located since it significantly decreased the neurogenic (post 30 min) algesia. A higher concentration of the extract (400mg/kg) was required for analgesia due to their central location, as shown in phase I of the formalin-induced pain test. In order to increase therapeutic concentrations in plasma, EEGA 200mg/kg showed maximum protective effect.

VIII. CONCLUSION

There have been many studies reporting that steroids and flavonoids produce analgesic effects. In addition, few studies have investigated the analgesic effects of tannin. The analgesic effect of *Gyrocarpus americanus* may be attributed to steroid, flavonoid, and tannin content. Analgesic activity of these plants may be attributed to their constituents.

The results showed that the ethanol extract of Gyrocarpus americanus Jacq. had significant analgesic activity in all the two models in a dose-dependent manner. The findings suggest that Gyrocarpus americanus Jacq. could be a potential source of natural analgesic compounds.

The study found that the extract exhibited significant analgesic activity in both the hot plate and formalin tests. Which further implied that the analgesic effect may be due to the presence of alkaloids in the extract.

The analgesic activity of the methanol extract of Gyrocarpus americanus Jacq. using the formalin test in rats. The study found that the extract produced a dose-dependent reduction in the number of flinches and licking behaviors in both the first and second phases of the test. These findings suggest that Gyrocarpus americanus Jacq. has significant analgesic activity in the formalin test and could be a potential source of natural analgesic compounds. However, further studies are needed to identify the active compounds responsible for the analgesic activity and evaluate their safety and efficacy in humans.

The analgesic activity of the methanol extract of Gyrocarpus americanus Jacq. using the hot plate test in rats. The study found that the extract produced a significant increase in the latency time for the animals to respond to the heat stimulus. These findings suggest that Gyrocarpus americanus Jacq. has significant analgesic activity in the hot plate test and could be a potential source of natural analgesic compounds. However, further studies are needed to identify the active compounds responsible for the analgesic activity and evaluate their safety and efficacy in humans.

IX. FUTURE PROSPECTIVES

Evaluation of analgesic activity of ethanol extract of Gyrocarpus americanus Jacqueline was done using the acetic acid-induced writhing test and the formalin-induced paw licking test on Swiss albino mice. The results showed a significant decrease in writhing episodes and paw licking time in a dose-dependent manner compared to the control group, indicating that the ethanol extract of Gyrocarpus americanus Jacqueline possesses analgesic activity.

The future scope of this study includes further investigation of the active constituents of the plant extract responsible for the observed analgesic activity using advanced techniques such as HPLC and LC-MS. Moreover, the mechanisms underlying the analgesic activity of Gyrocarpus americanus Jacqueline should be elucidated through possible involvement of opioid receptors or inhibition of prostaglandin synthesis pathways. Additionally, toxicity studies and clinical trials should be performed to determine the safety and efficacy of the extract for use in humans

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