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Invivo Analgesic Effects of Herbal Extracts on Animal Models.

Mehtab Malik***, Sheema Tarannum**
****(M. Pharmacy - Pharmacology*
Department of Pharmacology
Shadan Women's College of Pharmacy- Khairtabad-500004- Hyderabad)

Abstract:
Purpose: *To investigate the analgesic activity of the etahanolic extract of Chonemorpha fragrans and Cynometra ramiflora leaves in rats.*
Methods: *Hot plate, radiant source and acetic acid were used to induce pain in Wistar rats and Swiss Albino mice which were divided into six groups. The animal groups were thereafter administered CFEE (200 mg/kg), CREE (200 mg/kg), CFEE+CREE (200mg/kg) Aspirin (reference standard, 100 mg/kg) and normal saline (control), respectively. The pain was measured by observing the behavioral changes in rats like Licking of paw in case of Eddys Hopt Plate Method, Flicking of tail in Radiant Heat Method and Contractions of abdomen in Acetic acid induced Writhing. over a period of 2-3h. CFEE & CREE were also phytochemically screened for alkaloids, steroids, carbohydrates, tannins, fixed oils, proteins, triterpenoids, deoxy-sugar, flavonoid, cyanogenetic and coumarin glycosides.*
Results:*CFEE (200 mg/kg), CREE (200 mg/kg), CFEE+CREE (200mg/kg) significantly reduced analgesia ($p < 0.05$, $p < 0.01$, respectively). Phytochemical tests for Chonemorpha fragrans showed the presence of alkaloids, glycosides, carbohydrates, flavonoids, saponins, sterols, proteins. Cynometra ramiflora showed presence of alkaloids, carbohydrates, flavonoids, tannins, saponins, sterols, phenols. GCMS Analysis results showed presence of few chemicals which were responsible for analgesic activity. Histopathology of brain is also done which shows the effects of herbal extracts on brain tissues.*
Conclusion: *The etahanolic extract of Chonemorpha fragrans and Cynometra ramiflora leaves of possesses significant analgesic activity.*
Keywords*: Analgesic activity, Acetic acid, Chonemorpha fragrans and Cynometra ramiflora , Phytochemical screening, GCMS Analysis, Histopathology.*

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I. Introduction:

ANALGESIA: The failure to feel torment / pain. It is derived from the Greek word.
i.e., an = not; algein= feel pain.
Analgesia: PGs (Prostaglandins) induce hyperalgesia.
Role exhibited by Prostaglandins:
PGs seem to serve as in as algesic agents during irritation and inflammation. They cause delicacy and enhance the activity of different algesics. Inhibition of PG synthesis is a significant anti-inflammatory mechanism. Aspirin infused locally diminishes pain produced by infusion of bradykinin at the equivalent.

Mechanism of Action of Pain:
The pain pathway/ Pathophysiology:
- Pain receptors - (nociceptors) in the skin are enacted through tissue.
- A signal goes up the peripheral nerve to the spinal cord.
- Chemical messengers- (neurotransmitters) are released within the spinal cord which activate other nerves that pass signals to the brain.
- The thalamus transfers the signs on to the somatosensory cortex (sensation), frontal cortex (thinking) and limbic framework (enthusiastic/emotional reaction). **[1]**

When we experience pain for example when we touch a hot stove the nociceptors in our skin send signals through nerve fibres (A-delta fibres and C fibres) to Spinal cord, Brain stem and then to the Brain where it is registered sensation of pain, the information is thus processed and pain is perceived. **[2]**

II. Review of Drug Under Study:

Fig: 1. *Chonemorpha fragrans* Plant

Description: Chonemorpha fragrans belonging to the family Apocynaceae, Chonemorpha fragrans is a heavy spreading laticiferous bush with delicate grayish to corroded earthy colored bark which yields fiber of good quality; leaves straightforward, inverse, huge, orbicular, fulvous to mentose underneath, noticeably veined; blossom enormous, whitish to cream-yellow, fragrant, in terminal or pseudo-axillary cymose panicle; organic products long, straight, woody, equal, follicular mericarps; seeds some, level, right away hooked with long white satiny trance like state**[3]**

Biological Source:
- Evergreen timberlands and hallowed grooves in the field **[4]**
- Dense sloping territories, frequently sticking to trees**[5]**.
- Chonemorpha fragransis a restorative plant found in western ghats of Maharashtra. The leaves, roots, bark-stem are utilized in Ayurvedic arrangement of meds. Leaves are utilized as churna/separate or in blend with the other plant- material in their detailing. **[6]**

Chemical Constituents: The root bark contains 3.03 % of complete alkaloids present are japindine, N-formyl chonemorphin, N-methyl chonemorphin. Chonemorphin dihydrochloride is an antiamoebic principle and show in vitro action against parasites Entamoeba histolytica (25µg/ml) trichomonas vaginalis (200µg/ml) and invitro movement against hepatic amoebiasis in brilliant hamster and intestinal amoebiasis in wealing wistar rodents. Nearness of fats, octacosanol, ceryl liquor, β – sitosterol and taraxasterol is accounted for. The leavesand twig contain baurenolacetate and β-sitosterol. The stem yields latex. It is purgative. japindine, N-formyl chonemorphin, N-methyl chonemorphin. Nearness of fats, octacosanolceryl liquor, β – sitosterol and taraxasterol is accounted for. Leaves &twig contain baurenolacetate and β-sitosterol**[7]**

Morphological Characters: Chonemorpha fragrans is a bold spreading lactiferous bush with delicate greyish to corroded to earthy coloured bark which yields good quality fibre.
Leaves- simple, large, opposite, fulvous tomentose underneath, orbicular, veined prominently.
Flowers- large,fragrant, whitish to creamy-yellow, in pseudo-axillary or terminal cymose panicle.
Fruits- straight, long, parallel, woody, many seeds, flat, shortly bent with white long silky coma, mericarp is follicular.

Taxonomy/Scientific Classification:[7]	**Vernacular Names: [7]**
Kingdom: Plantae Phylum: Division Class: Angiospermae Order: Gentianales Family: Apocynaceae Genus: Chonemorpha Species: Chonemorphafragrans	Hindi: Garbhedaro Telugu: Chaga Sanskrit: Murva, Morata Kannada: Manjinaru Malayalam: Perunkurumpa

Theraputic Activities: [7]
- Antidiabetic effect
- Antipyretic activity
- Anti-parasitic effect

- Skeletal muscle relaxant
- Anticancer action
- Anthelmintic action
- Gynaecological confusion
- Antibacterial Activity
- Antioxidant potential and DNA assurance capacity

Traditional Uses: The roots act as astringent, laxative, sweet, thermogenic, bitter, expectorant, depurative, digestive, antiscorbutic, carminative, anthelmintic, anodyne and febrifuge. These have been useful in vitiate diseases, scabies, leprosy, dyspepsia, constipation, colic, hyperacidity, cardiac debility, diabetes, cough, jaundice, bronchitis & intermittent fevers. Murva is utilized in diseases like anaemia(pandu), fever (jwara), diabetes (prameha), stomach disorders (udararoga), typhoid (visamajwara), urinary infections (asmari) and cough (ksaya) .its also utilized in curingdiarrhoea, polyuria, boils, Leprosy, eye diseases, vomiting and poisoning conditions of kapha&vata, skin diseases, scabies, leprosy, dyspepsia, hyperacidity, jaundice, cough, bronchitis and intermittent fevers.

Fig: 2.*Cynometra ramiflora* Plant

Description: Cynometra ramiflora belongs to family Fabaceae,It isa tree, up to 26 m tall. Its crown is adjusted and umbrella fit as a fiddle. Its substitute, followed, pinnate leaves have 1-2 sets of pamphlets that are 1.2-20 by 0.5-7 cm. New foliage is pinkish to beige in shading. **[8]**

Biological Source:A tree in the family Fabaceae, Cynometra ramiflora is foundin mangroves and overwhelmed timberlands from New Caledonia in the western Pacific west to Queensland in Australia, New Guinea, Island Southeast Asia, and Tropical Asia as far west as India. Its wood is utilized for development and fuel, and parts of plant are credited therapeutic use.**[9]**

Chemical Constituents:

Roots are considered as purgative and laxative.

- Ethanolic remove yielded three mixes as significant constituents, viz., caffeic corrosive (1), apigenin (2) and 3-(2,3,4-trihydroxyphenyl)- 7-hydroxycoumarin (3).
- Leaves yielded flavonoids, tannins, alkaloid, phenolics, saponins &steroids. **[9]**

Morphological Characters:

Leaf- with 2 pairs of leaflets, pointed emerginate tip.

Flowers: white to creamy yellow, with stigma and style- straight, ovary curly hair outside and glabrous inside.

Fruits: clusters, with sub-terminal beak, mature fruits.

Dry scaly bark and dark coloured seeds.

Taxonomy/Scientific Classification:[9]	**Vernacular Names: [9]**
Kingdom: Plantae (unranked): Angiosperms (unranked): Eudicots (unranked): Rosids Order: Fabales Family: Fabaceae Genus: Cynometra Species: C. Ramiflora	Hindi: sinthomra raamiphlora Kannada: kanaga, kanaka Malayalam: irappa, irippa, irupa Tamil: irapu, irutpu, naipudukan, nay putukkan, naypputukkan Bengali: Shinguri, Shingar, Singra, Shingra, Seeri MALAYSIA: Katong laut. INDONESIA: Kateng, Kepel, Sala, Wunut. AUSTRALIA: cynometra, wrinkle pod mangrove.

Theraputic Activities:[9]
- Cytotoxic/Anticancer Activity
- Blood Glucose Lowering
- Neuropharmacological/Antibacterial/Antinociceptive
- Antioxidant
- Xanthine Oxidase Inhibitory Activity/Leaves
- Antibacterial/Stem Bark
- Anti-Ulcer/Leaves
- Glucose Lowering/Stem Bark

Traditional Uses: [9]
- Roots are purgative.
- Seeds & leaves are utilized as anti-herpetic.
- In Malabar, leaves are utilized to make lotions for skin ailments/diseases.
- Oil extractedfrom seeds are utilized for skin infections.
- In Bangladesh, leaves are utilized to make honeyed lotion & boiled in cow's milk is applied to scabies, leprosy, and different skin lesions. Seed oilis also utilized for same purpose.
- In Indonesia, hypertension, high uric acid & diabetes istreated by using plants. **[10]**

III. Materials and Methods:

Methadology:

Collection of Plant: The driedleaves of both plants Chonemorpha fragrans (Moon) Alston family Apocynaceae and Cynometra ramiflora family Fabaceae were collected, which are taxonomically identified and authenticated by Dr. K Madhava Chetty, Assistant Professor of Botany, Department of Pharmacognosy, Sri Venkateshwara University, Tirupathi.

- **Materials Required:**
- **Plants:** Chonemorpha fragrans and Cynometra ramiflora plant leaves powder.
- **Animals required:** Male Albino Wistar rats weighing 150-200 gm.
- **Chemicals and Reagents:**
- Normal saline (0.9% w/v) - utilize as solvent to dissolve the test and standard drugs.
- Aspirin (20mg/kg) - standard drug for analgesia.
- Ethanol (99% v/v) preparation of plant extracts.
- All chemicals & reagents of analytical grade are used for present research.
- **Preparation of Plant Extract:**

The leaves should be sliced into pieces and exposed to shade drying. On absolute drying, the pieces should be powdered and put away in impermeable holders at room temperatures. The powdered leaves will be macerated with ethanol upto 7 days &afterwards separated. The filtrate will be dissipated to acquire dried concentrate. The extract obtained will tested for analgesic, anti-inflammatory and anti-pyretic activites. The plant extricate will be made by maceration process. **[11]**

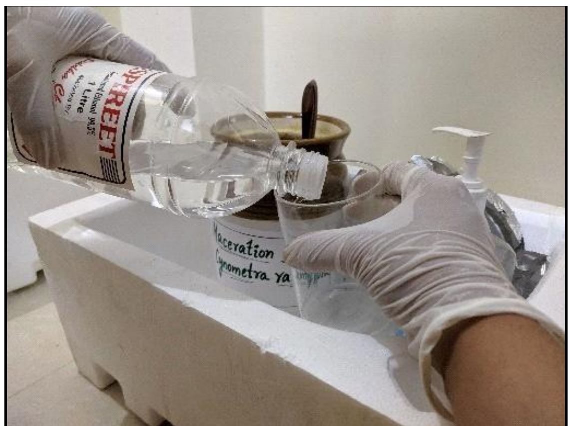


Fig: 3. Preparation of Plant Extract

Maceration: In maceration (for liquid extract), entire or coarsely powdered plant-sedate will be placed incontact with dissolvable in a stoppered compartment for a characterized period with visit unsettling until

solvent issue is disintegrated. This technique is best reasonable for use if there should be an appearance of thermolabile medications. Utilizing this procedure, 500g/kg powder will be included, ethanol in the proportion 1:2 with fiery shaking was completed for 7 days consistently and was kept at room temperature. The filtrate in this manner will be obtained which is the ethanolic extricate. The filtrate got will be broken up in 0.9% ordinary saline which will be utilized as vehicle later on tests.**[11]**

Fig: 4. Maceration

Experimental Animals:

Male Albino Wistar rats weighing 150-200 gm will be used. The experimental animals shouldbe maintained under std laboratory conditions (22-28℃, 12-h light/dark cycle under controlled temperature.) All animals should be acclimatized to the laboratory environment for not less than one week before the commencement of experiment.**[11]**

Fig: 5. Experimental Animals

Acute Toxicity Studies: Acute Toxicity Studies is performed according for the extract as per the intense harmful exemplary strategy according to Litchfield and Wilcoxon (1949) which is an acute toxic classic method. Intense poisonousness study will be conveyed out on plant extricates utilizing Male Albino mice in future. The mice must be fasted overnight and just before the starting of experiment, weight of mice is recorded. The animals has to be separated into five groups containing 6 animals each, the extract will be given orally in expanding portion up to 2000mg/kg b.w. After treatment animals will be watched for toxicity or mortality for 72 hours. No

adjustments in skin and hide, eyes, autonomic (salivation, lacrimation, poop) and central sensory system (ptosis, laziness, step, tremors) should be observed.**[11]**

Phytochemical Screening: Preliminary phyto-chemical screening will be performed according to the standard methods. The ethanolic leaf extracts of C. fragrans (C.F) and C. ramiflora (C.R) will be screened/tested for the presence of various phytochemicals like -Alkaloids, Flavonoids, Glycosides, Steroids, Terpenoids, Anthraquinones, Proteins, Phenols and Anthocyanins by employing standard conventional protocols. **[11]**

Fig:6. Ethanolic extract & Phytochemical Testing

Screening Methods: [12]

Analgesic Activity: Pain is a side effect of numerous diseases requiring treatment with analgesics. Serious pain because of cancer metastases needs the utilization of analgesics which are strong, that implies opioid medications. The dependence obligation of opioids prompted serious exploration for compounds without adverse drug reactions. Numerous methodologies have been utilized to separate the different activities of strong analgesics by developing animal models for analgesic activity as well as for addiction liability. Many kinds of opioid receptors have been recognized in the brain permitting in vitro binding tests. However, the in vitro tests partially can substitute for animal tests including pain (torment). Pain is a typical wonder in all creatures, in any event in vertebral animals, like that felt by man. Pain relieving impacts in animals are equivalent with the therapeutic impacts in man. Obviously, that in each example painful stimuli to animals must be limited however as much as could reasonably be expected. Painful stimuli can comprise of direct incitement of the efferent sensory nerves or incitement of pain receptors by different methods, for example, heat or pressure. The function of endogenous peptides, for example, enkephalins and endorphins gives more understanding into brain processes and the activity of central analgesics.

In vivo methods for testing central analgesic activity

1.) Hot plate method

PURPOSE AND RATIONALE: The paws of rats and mice are very heat sensitive at temperatures which do not damage the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The time until these reactions happen is delayed after administration of centrally acting analgesics, though peripheral analgesics of the acetylsalicylic acid or phenyl-acidic acid type doesn't generally influence these reactions.

PROCEDURE: The technique initially portrayed by Woolfe and Mac Donald (1944) has been adjusted by a many investigators. The accompanying alterations has been demonstrated to be reasonable: Groups of 10 mice of either sex with an underlying load of 18 to 22 g are utilized for each portion. The hot plate, which is commercially accessible, comprises of an electrically heated surface. The temperature is controlled for 55° to 56 °C. This can be a copper plate or a hot glass surface. The animals are put on the hot plate and the time until either licking or jumping happens is recorded by a stop-watch. The dormancy is recorded when 20, 60 and 90 min following oral or subcutaneous administration of the standard or the test compound.

Fig: 7. Hot plate method

EVALUATION: The prolongation of the dormancy times looking at the qualities when administration of test compounds or the estimations of the control with the test groups can be utilized for statistical correlation utilizing the t-test. Whereas, the qualities which surpass the values before administration for half or 100% can be viewed as certain and ED50 qualities can be determined. Portions of 7.5 mg/kg s.c. morphine hydrochloride, 30 mg/kg s.c. codeine hydrochloride, 30 mg/kg s.c. pethidine hydrochloride and 400 mg/kg s.c. phenazone were discovered to be powerful, though anti-inflamatory medicine demonstrated no impact even at high dosages.

2.) Radiant Heat Method

PURPOSE AND RATIONALE: For evaluation of analgesic activity of opiates an quantitative measurements of pain threshold in man against thermal radiation, Schumacher et al. (1940), Wolff et al. (1940) developed a method which was later followed by many authors to study analgesic properties in animals.

This was done by measuring the changes caused by administration of drug in the sensitivity of mice/rats, to heat stress applied to their tails. This test is very helpful to distinguish non-opiate analgesics and centrally acting morphine-like analgesics.

Mice are caged with their tail exposed to a light beam focused on the proximal third of the tail. The time taken by the mice to react is measured.

PROCEDURE: The method was described by Ther, Lindner and Vogel (1963) as a modification of earlier publications (D'Ar-mour and Smith 1941).

For each dose, a group of 10 mice with NMRI strain are used with weight ranging from 18 - 22 gm of either sex. The normal reaction time is noted before the standard or the test compound is given to the animal.

The animal is cage with an exposed tail which is held by the investigator. Again, a light beam is focused on the proximal third of the tail. This is continued till the mice gives a reaction like either tries to pull the tail and turn the head. The time is measured and the mice with a reaction time of more than 6 seconds are disregarded.

The escape reaction is observed as a complex phenomenon mediated by the animal's brain but only tail flicking movements are regarded as spinal reflexes. Therefore, the influence of the drug on the brain can be truly assessed by the observation of the escape reaction.

The administration of test compounds and the standard is either done orally or subcutaneously.

These tests are performed after 30, 60 and 120 minutes and the reaction times are noted individually for each animal.

Fig: 8. Radiant Heat Method

EVALUATION: Evaluation can be done in 2 possible ways,
Firstly, we compare the pretest values with average values of the reaction time after each time interval.
The animals with double or more than double the reaction time as the pretest values are considered positive. For each time interval and each dose, % of positive animals are counted and the ED50 values are calculated according to LITCHFIELD and WILCOXON.

3.) Writhing tests
PURPOSE AND RATIONALE : Pain is actuated by infusion of irritants into peritoneal cavity of mice. Animal respond by a reaction called stretching or writhing. The test is reasonable to recognize analgesic action albeit some agents that are psychoactive additionally show action. An irritatinf reagent for example, phenylquinone or acitic acid is infused intraperitoneally to mice and the wriuthing response is assessed. The response isn't explicit for the irritant.

PROCEDURE : Mice with weight ranging between 20 to 25 gm of either sex are used. A 0.02% concentration of Phenylquinoneis suspended in a 1% carboxymethylcellulose suspension. A 0.25 ml aliquot of this suspension is injected intraperitoneally. 6 animals were grouped and used for treated mice and controls. 2 groups of 6 mice were preferred to be used as controls. At various pretreatment times, the test animals were administered to the drug or the standard, before administering with phenylquinone. For the next 5 minutes, mice were introduced into glass beakers and then further observed for 10 minutes, and the number of writhes were recorded individually for each mice. A writhe is indicated by stretching of abdomen while simultaneously stretching at least on hind limb. This is done for the scoring purpose. The percent inhibition formula is : Average writhe in control group minus writhes in drug group divided by the writhes in the control groups, times 100%. The peak time is considered based on greatest % of inhibition. A dose range is reserved for interesting compounds or those with more than 70% writhing. Compounds with minimal activity were the one with less than 70% inhibition.

Fig: 9. Writhing test

EVALUATION: Dosage run is in accordance to the response but at the peak of drug activity, 8 animals were tested. A vehicle control group and 4 drug groups were employed. Testing and dosage in animals were done randomly and estimated ED50 is calculated. The values for ED50 were found to be 1.0 mg/kg p.o. indomethacin, 30 mg/kg p.o. acetylsalicylic acid and 40 mg/kg p.o. phenacetin.

Experimental Design: The animals (Albino Wistar rats) will be divided into 6 groups, each group containing 6 rats (n=6). Total – 60 rats.

Groups	Drugs	Dose & Route
Group 1	Normal Saline	1 ml - i.p
Group 2	Toxic Control	10ml/kg - i.p
Group 3	Standard Control	10ml/kg -i.p
Group 4	CF Leaf Extract	200mg/kg - po
Group 5	CR Leaf Extract	200mg/kg - po
Group 6	CF + CR Leaf Extract	200mg/kg - po

IV. Results:

Phytochemical Evaluation: After successful Ethanolic leaf extracts of Chonemorpha fragrans and Cynometra ramiflora by Maceration process the following Phytochemical analysis was done and are summarised inthe following Table.

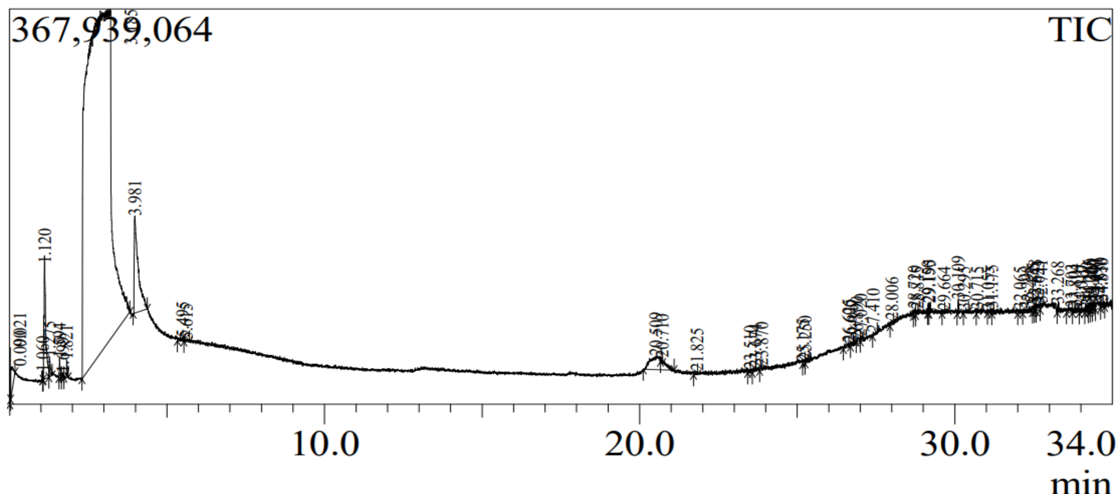
Table 1: Phytochemical Analysis of C. Fragrans & C. Ramiflora:

Type of constituents	C.F ethanolic leaf extract	C.R ethanolic leaf extract
Alkaloids	+	+
Glycosides	+	_
Carbohydrates	+	+
Flavonoids	+	+
Tannins	_	+
Saponins	+	+
Sterols	+	+
Phenols	_	+
Proteins	+	_
Triterpenoids	_	_

+ indicates presence and **-** indicates absence.

GCMS ANALYSIS:

GCMS of Ethanolic Extract of Chonemorpha fragrans:



Peak Report:

S.no	Retention time	Chemical constituents	Area %	Uses
1	20.509	Dimethyl Sulfoxide	1.75	Decreases pain and inflammation.
2	22.450	Sulfamide	1.02	Analgesic and Anti-inflammatory, Treats severe burns.
3	29.150	Phenol	0.05	Used as an oral analgesic, relieve itching, treats pharyngitis.
4	32.760	Butyric acid	0.20	Treats pain and inflammatory conditions (non-specific bowel inflammation, diverticulitis, diversion colitis)
5	23.870	2-Propanol	1.34	Used to prevent migraine headaches and chest pain (angina)
6	25.175	Propanoic acid	1.02	Treatment of fever and inflammation associated with tissue injury.

GCMS of Ethanolic Extract of Cynometra ramiflora:

Peak Report:

S.no	Retention time	Chemical constituents	Area %	Uses
1	1.055	1,1-Cyclopropanedicarbonitrile	0.07	Decreases pain
2	20.309	Dimethyl Sulfoxide	2.40	Treats painful bladder syndrome, decreases topical pain.Treats inflammation, headache, osteoarthritis, rheumatoid arthritis, severe facial pain.
3	32.625	Hydroxybutyric acid	0.07	Analgesic
4	29.365	phenothiazone 32	0.09	Treats moderate to severe pain.
5	31.055	Pyridine-2	1.02	Relieve symptoms caused by irritation of the urinary tract such as pain, burning, and the feeling of needing to urinate urgently or frequently.
6	34.20	Dimethoxyamine	2.80	Analgesic, Anti inflammatory.

INVIVO Models for Analgesic Activity:

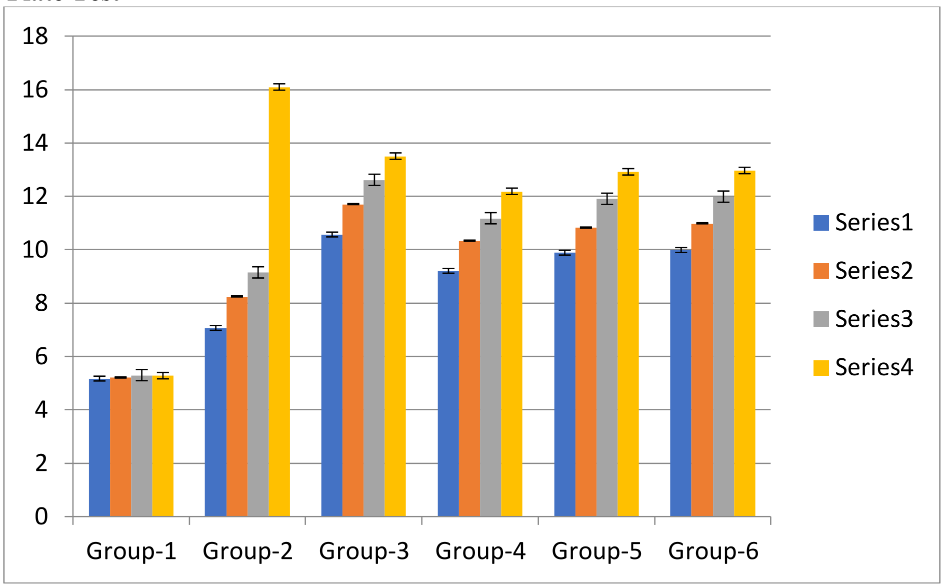
Eddy's Hot Plate Test:

TREATMENT	REACTION Initial Time	TIME		
		15 minutes	30 minutes	60 minutes
Normal Control	5.16 ± 0.20	5.20 ± 0.28	5.29 ± 0.30	5.27 ± 0.29
Toxic Control	7.06 ± 0.378	8.24 ±0.420	9.14 ± 0.430	16.09 ± 0.392
Standard	10.56 ± 0.253***	11.7 ±0.262***	12.61 ± 0.261***	13.50 ± 0.260***
CFEE	9.20 ± 0.460**	10.33 ± 0.372**	11.17 ±0.462**	12.18 ± 0.459**
CREE	9.88 ± 0.184*	10.82 ± 0.182*	11.90 ± 0.187*	12.91 ± 0.186*

CFEE + CREE	9.98 ± 0.178***	10.98 ± 0.241***	11.98 ± 0.182***	12.96 ± 0.182***

Data is indicated by MEAN ± SEM,
*Statistically significant comparing to Toxicant group(II) at $p<0.05$;
**Statistically significant comparing to Toxicant group(II) at $p<0.01$;
***Statistically significant comparing to Toxicant group(II) at $p<0.001$;

Eddy's Hot Plate Test

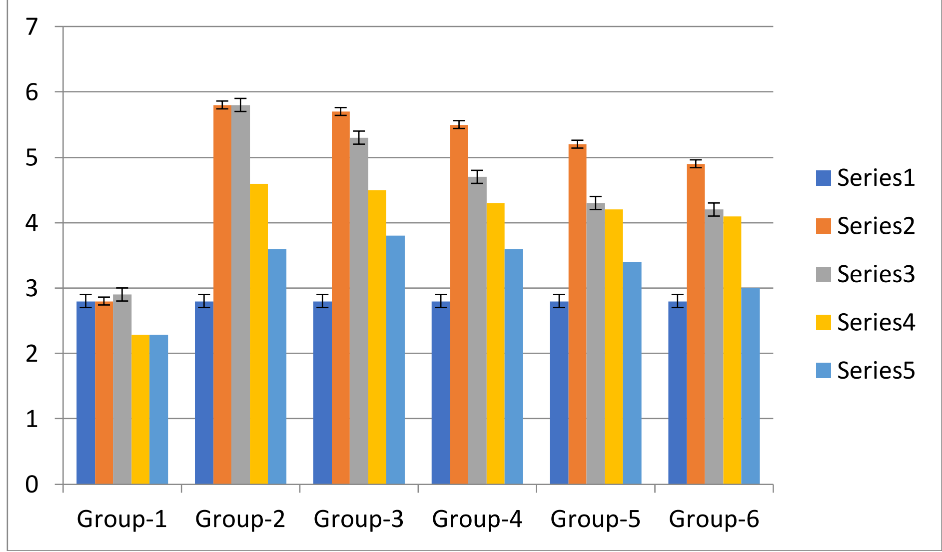


Graph -1. Effect of CFEE and CREE on rats induced on Hot Plate

Radiant Heat Method:

TREATMENT	Time				
	0 min	**30 min**	**1 hr**	**2 hrs**	**3 hrs**
Normal Control	2.8 ± 0.06	2.8 ± 0.14	2.9 ± 0.15	2.29 ± 0.18	2.29 ± 0.13
Toxic Control	2.8 ± 0.11	5.8 ± 0.10	5.8 ± 0.17	4.6 ± 0.11	3.6 ± 0.12
Standard	2.8 ± 0.12	5.7 ± 0.12	5.3 ± 0.08	4.5 ± 0.08	3.8 ± 0.11
CFEE	2.8 ± 0.11	5.5 ± 0.8	4.7 ± 0.06	4.3 ± 0.16	3.6 ± 0.08
CREE	2.8 ± 0.16	5.2 ± 0.17	4.3 ± 0.02	4.2 ± 0.08	3.4 ± 0.05
CFEE + CREE	2.8 ± 0.17	4.9 ± 0.11	4.2 ± 0.01	4.1 ± 0.01	3.0 ± 0.01

Radiant Heat Test



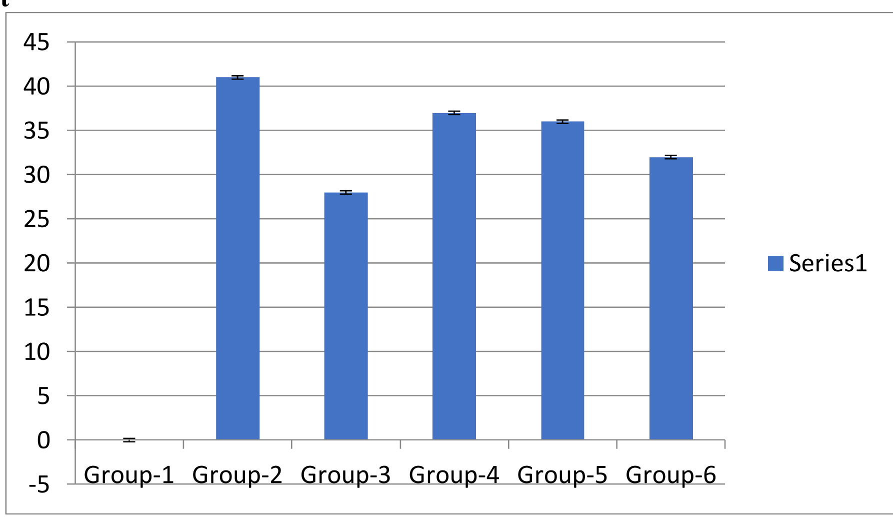
Graph -2. Effect of CFEE and CREE on application of Radiant heat

Writhing Test:

Treatment Groups (n=6)	No. Of Writhigs (in 10 mins)
Normal Control	0
Toxic Control	41 ± 0.67
Standard	28 ± 0.78***
CFEE (200 mg/kg)	37 ± 0.48*
CREE (200 mg/kg)	36 ± 0.78**
CFEE + CREE (200 mg/kg)	32 ± 0.75***

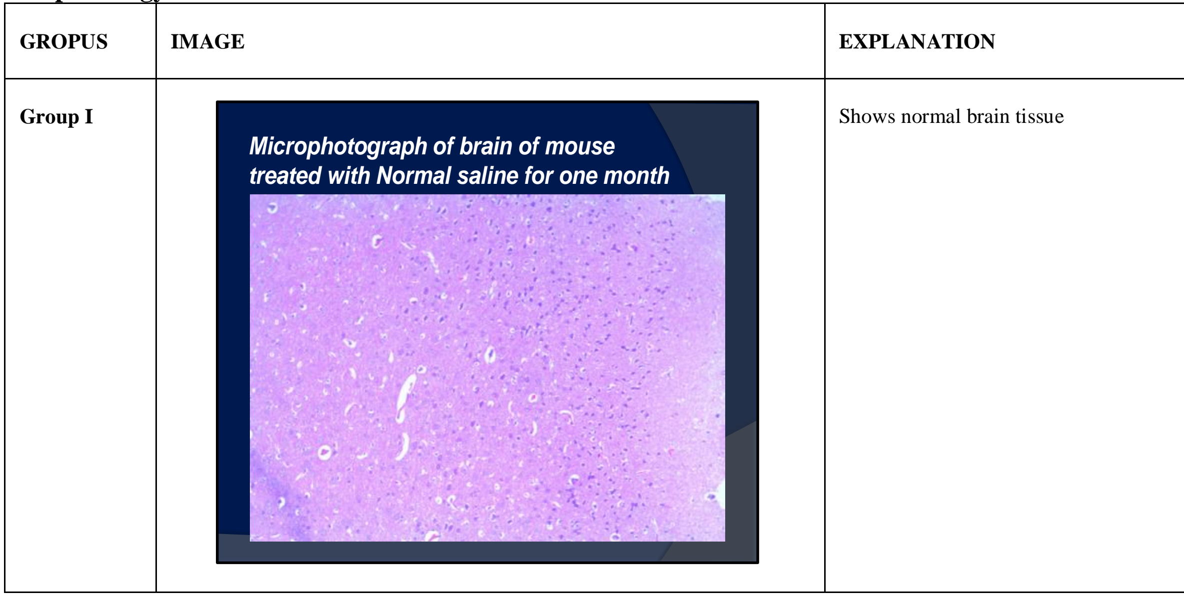
Data is indicated by MEAN ± SEM,
*Statistically significant comparing to Toxicant group(II) at $p<0.05$;
**Statistically significant comparing to Toxicant group(II) at $p<0.01$;
***Statistically significant comparing to Toxicant group(II) at $p<0.001$;

Writhing Test

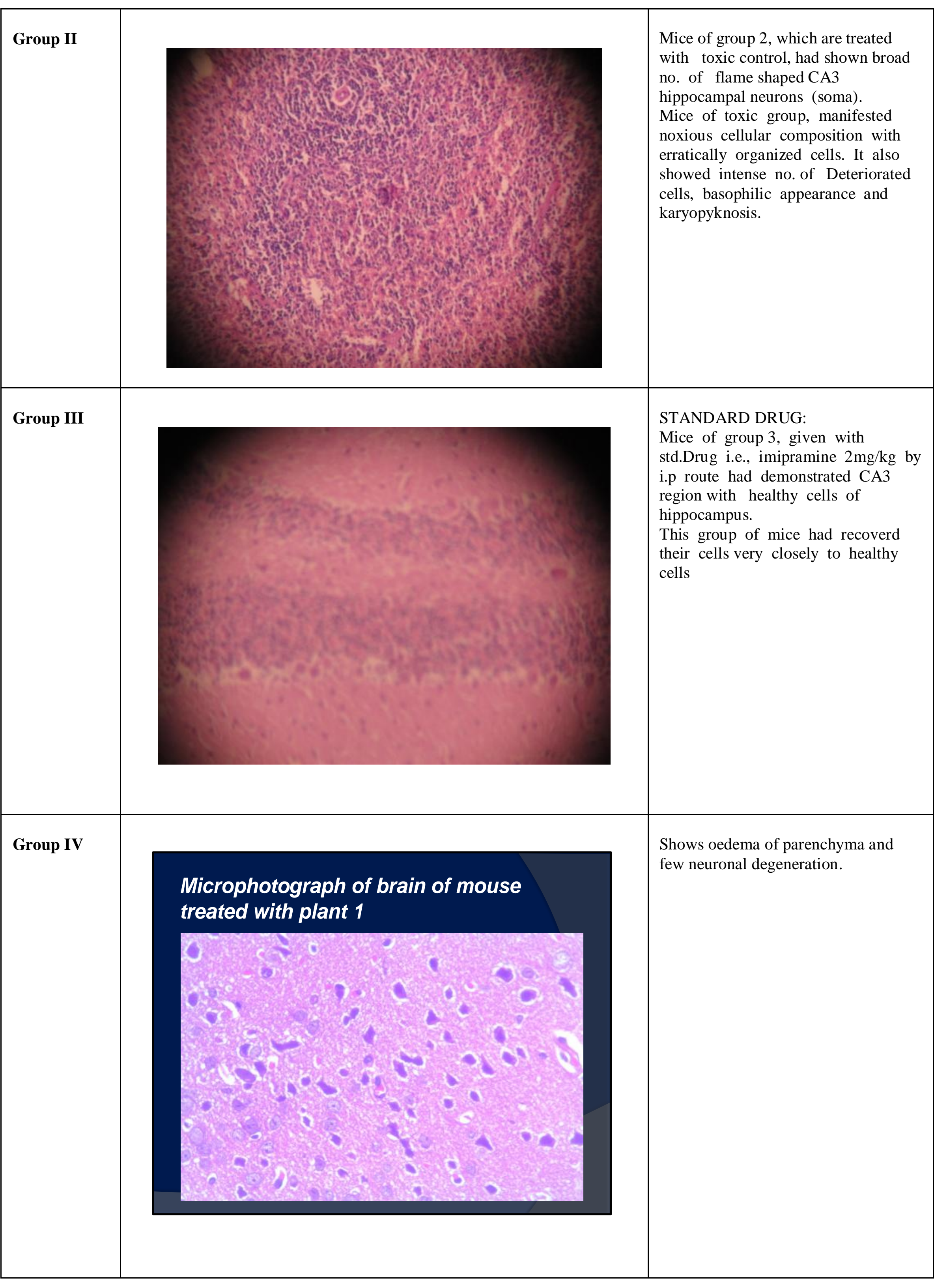


Graph -3. Effect of CFEE and CREE on abdomen of acetic acid induced rats.

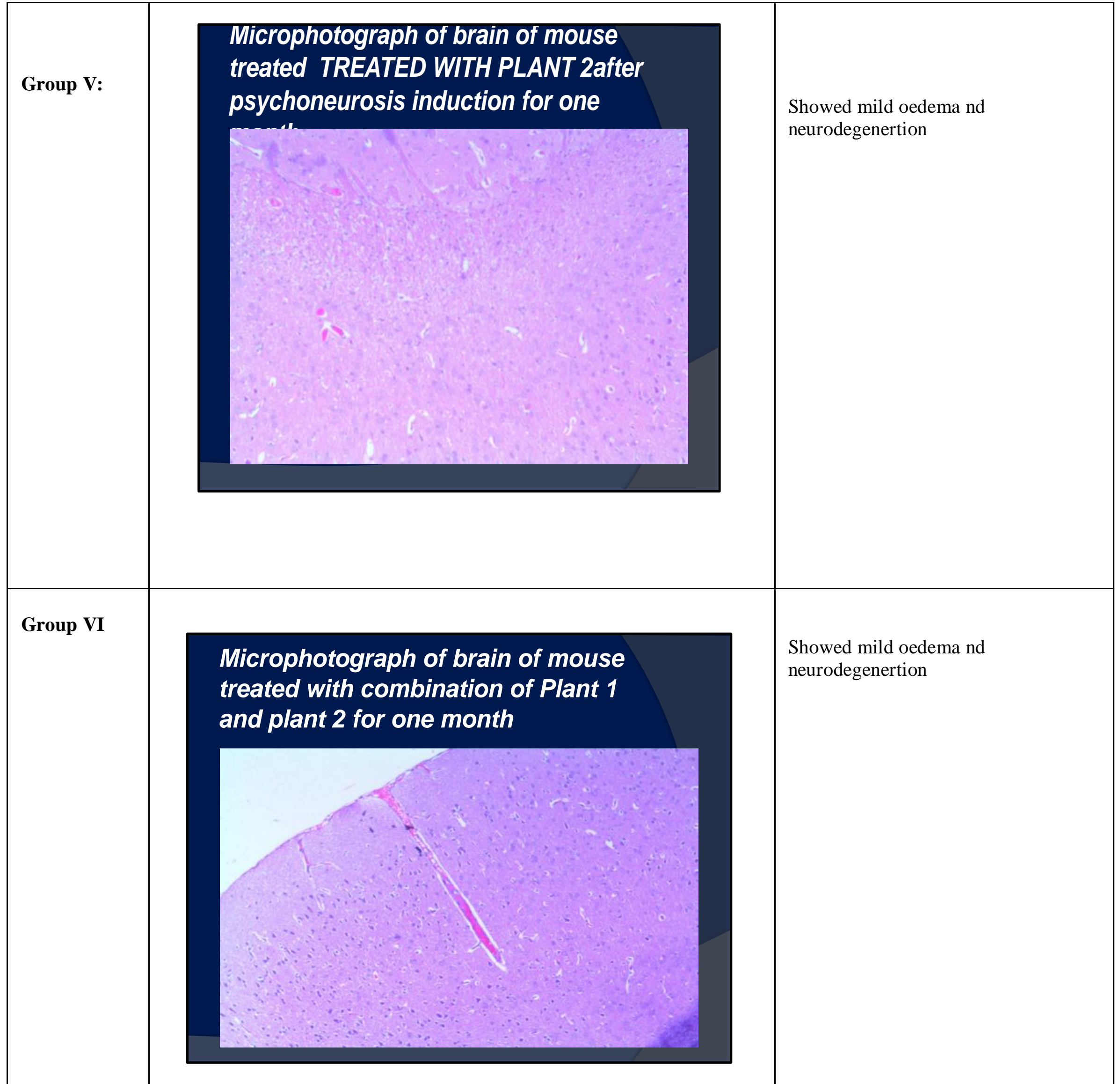
Histopathology:



GROPUS	IMAGE	EXPLANATION
Group I		Shows normal brain tissue



Group II		Mice of group 2, which are treated with toxic control, had shown broad no. of flame shaped CA3 hippocampal neurons (soma). Mice of toxic group, manifested noxious cellular composition with erratically organized cells. It also showed intense no. of Deteriorated cells, basophilic appearance and karyopyknosis.
Group III		STANDARD DRUG: Mice of group 3, given with std.Drug i.e., imipramine 2mg/kg by i.p route had demonstrated CA3 region with healthy cells of hippocampus. This group of mice had recoverd their cells very closely to healthy cells
Group IV		Shows oedema of parenchyma and few neuronal degeneration.



Group V:		Showed mild oedema nd neurodegenertion
Group VI		Showed mild oedema nd neurodegenertion

V. Discussion:

My selected extracts from *Cynometra ramiflora and Chonemorpha fragrans* are effective singularly and also when used together.
Eddy's hot plate method had been performed and chonemorpha fragrans + cynometra ramiflora was effective up to 98% while chonemorpha fragrans is 82% effective and cynometra ramiflora is 88% effective.
Radiant heat method was performed and chonemorpha fragrans was effective up to 90% while cynometra ramiflora was effective up to 85% while chonemorpha fragrans + cynometra ramiflora was effective up to 80%.
Writhing test had been performed and chonemorpha fragrans + cynometra ramiflora was effective up to 99% and chonemorpha fragrans was effective up to 86% and cynometra ramiflora was 82% effective.
All the effects were compared against standard group.

VI. Conclusion

The ethanolic extricates of Chonemorpha fragrans and Cynometra ramiflora has exhibited promising Analgesic, activity because of the presence chemical constituents found out by phytochemical screening.

The alkaloidsare important as those are natural products with wide range of medicinal properties including pain relief, analgesic which are present in Chonemorpha fragrans and Cynometra ramiflora. They bind to nociceptors and either activate or inactivate them &inhibit or activate ion channels thus decreasing pain.

The flavonoid has been established to modify pain reducingreaction by inhibiting release of prostaglandins, a compound that results in pain, thus reducing pain.

Therefore, the plan of work was successful and was utilized inanimal studies & also helpful in treating analgesiaand other associated complications.**[13]**
Thus, this study was conducted toinvestigate the phytochemicals in Chonemorpha fragrans and Cynometra ramiflora and for conducting animal studies to treat Analgesia either by utilizing the plant individually or in combination on Male - Albino Wistar rats.
Medicinal plants & their chemical constituents have been widely utilized for pain relieving i.e., analgesics properties. They have their roles in prevention &treatment of analgesia.
In this perspective the Ethanolic extricates of Chonemorpha fragrans and Cynometra ramiflora and their chemical constituents were investigated for having properties to treat this ailment with the helpof GCMS Analysis and Preliminary phytochemical screening.

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