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INVESTIGATION FOR ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES USING ETHANOLIC ROOT EXTRACT OF ACONITUM ATROX AND LEAF EXTRACT OF CONIUM MACULATUM ON ANIMAL MODELS

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Abstract:
The assertion of the pharmacology of natural product is to explore benefits of natural resources for the mankind. Drugs extracted from natural resources are considered as primary source for drug discovery. Thus, the current study was designed to evaluate the safety profile and explore the analgesic and anti-inflammatory activity of ethanol extract of root of Aconitum atrox and leaf of Conium maculatum. They have been used for several therapeutic purposes. Eddy's hot plate methods was used to assess analgesic activity while anti-inflammatory activity was evaluated by carrageenan induced paw edema method. The root extracts of A. atrox and leaf extractof C. maculatum were found to be safe and showed significant analgesic and anti-inflammatory activity in comparison with the control group. The therapeutic effects of these extracts were almost comparable to aspirin and indomethacin. Therefore, their root and leaf can be used as effective analgesic and anti-inflammatory agents.

Keywords: *Analgesic activity, Anti-inflammatory activity, Extract, Herbal medicine, Aconitum atrox and Conium maculatum, Eddy's hot plate, Carrageenan induced paw edema, cox-assay methods.*

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INTRODUCTION:

1.1 Introduction to herbal medicine: Healing with herbal medicine is as old as mankind himself. Various sources known for using weed plants is the result of years of struggle against diseases that led man to learn to pursue drugs in the form of fruits, trunks, leaves, flowers, blisters, seeds, stem, and other parts of plants. (1) Many traditional medicines come out of plant sources a century ago. Modern Western herbal system highlights the impact of weeds on individual physical systems. For example, herbal medication might be used for their meant hemostatic, anti- inflammatory hemostatic, anticipated hemostatic, or immune stimulatory residences. Herbal flora differs from conventional drugs which might be typical as an uncouth extract, a combination of weed and diagnostic rules are based totally on the treatment of "root causes". (2)

1.2 Definition of disease:

1.2.1 Analgesic: It is defined as a substance that reduces pain by selectively acting on CNS and on PNS without loss of consciousness.

1.2.2 Pain: *Algesia* is a Latin word that means pain, it is an ill-defined, obnoxious sensation, usually evoked by an external or internal noxious stimulus. Pain is a warning signal, which results in uneasiness and suffering and may even be unbearable and incapacitating. (3)

1.2.3 Types of pain: Pain can be classified as

Nociceptive pain: Nociception utilizes interchangeably with nociperception is the relation of our bodies' sensorynervous system with regard to actual or potentially injurious incentive.

Neuropathic pain: It is usually suggested as a nerve laceration or nerve incapacity and is regularly correlated withallodynia.

Inflammatory pain: Infection is an instinctual organic stimulus produced by the tissues inside our bodies as a response to the danger incentive in an effort to get rid of the necrotic cells an initiate the tissue repairing technique.

1.2.4Anti-inflammatory: An anti-inflammatory is the property of a substance or treatment that eases inflammation or swelling. Anti-inflammatory drugs are called as anti-inflammatories, male about half of analgesics. These drugs reduce inflammation as opposed to opioids, which affect the central nervous system and block pain signals to thebrain. (4)

1.2.5 Inflammation: Defined as the local response of living mammalian tissues to injury due to any agent. It is a body's defense reaction in order to eliminate or limit the spread of injurious agents followed by the removal of the necrosed cells and tissues. (5)

1.2.6 Inflammation types: Depending upon the protection capacity of the host and length of reaction irritation can be divided as acute and chronic. Acute inflammation: It is of short duration lasting less than 2 weeks and represents the early body reaction, resolves quickly and is usually followed by healing. Chronic inflammation: Chronic inflammation is a long term and arises either after the agent has long resided due to severe inflammation or the initiation is such that it affects chronic inflammation from the beginning. (6)

1.3 Pathogenesis:

In and about the inflamed tissue, there is buildup of oedema fluid in the interstitial compartment which comes from blood plasma by its escape through the endothelial wall of peripheral vascular bed. In the initial stage, the escape of fluid is because of vasodilatation and resulting raise in hydrostatic pressure. This is transudate in nature. But later, the characteristics inflammatory oedema, exudate, appears by increased vascular permeability of microcirculation.

1.4 Agents causing inflammation:

1. Infective agents like bacteria and their toxins, fungi, and parasites
2. Immunological agents like cell-mediated antigen antibody reaction
3. Physical agents like heat, cold, radiation, and mechanical trauma
4. materials such as foreign bodies.

1.5 Signs of inflammation:

The Roman writer Celsus in the 1st century A.D. named the famous 4 cardinal signs of inflammation as: *rubor* (redness); tumour (swelling); *calor* (heat); and *dolar* (pain). To these, the fifth sign *function laesa (*loss of function) was later added by Virchow. The word inflammation means burning. This nomenclature had its origin in old times but now we know that burning is only one of the signs of inflammation. [5]

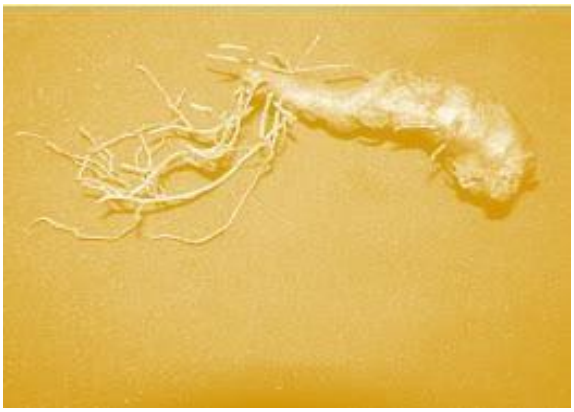
1.6 Aanti-inflammatory drugs:

1. Salicylates and their congeners.
2. Para- Aminophenol derivatives e.g., phenacetine, paracetamol.
3. Pyrazolone derivatives e.g., phenyl- butazone and oxyphenbutazone.
4. Heterocyclic aryl acetic acids derivatives e.g., Diclofenac
5. Propionic acid derivatives e.g., Ibuprofen, Naproxen. [7]

2. REVIEW OF DRUGS:

2.1 Introduction of plant-1

Plant name: Aconitum atrox.



(A) Description and Morphology: Population of Aconitum atrox were scrutinize in varying geographical locationsof the Garhwal Himalayas for studying morphological and biochemical variations. Different populations were noted to have significant differences in leaf characteristics such as length, width, biomass, fresh and dry weights of the shoot, length and diameter of the tubes. This species exhibits considerable polymorphism in morphology and biochemical attribute in relation to different habitats. (8)

(B) Chemical constituents: Tubers of A. balfourii Stapf mainly contain a crystalline toxic alkaloid called pseudo aconitine (0.4 to 0.5%) and aconitine in small amounts. Pseudacontine is a diterpene alkaloid, with the structural formula C36H51NO12. The crystal melts at 202°C and is moderately soluble in water, but more soluble in alcohol. When heated in a dry state, it undergoes pseudaconotine pyrolysis and formed pyropseudaconitine C34H47O10N. (9,10)

(C) Taxonomy: Kingdom: Plantae, Class: Magnoliopsida, Family: Ranunculaceae, Genus: Aconitum, Species: balfourii Stapf. (11)

(D) Vernacular names: Balfour's Monkshood, Gobaree, Meetha, Meetha jari, Mithabish. (12)***Therapeutic uses:*** It is used as antimicrobial, antibacterial, cytotoxic, anti-epileptiform, arrhythmogenic agents. It is also used in the treatment of various diseases such as arthritis, fever, rheumatism, leprosy, cough, sedative, anti-pyretic, febrifuge, vermifuge. (13,12)

2.2 INTRODUCTION OF PLANT-2

Plant name: Conium maculatum

(A) Description and Morphology: Conium maculatum is a tall, branched plant with white flowers. It is a biennia plants, usually 120-180(200) cm high. The root, described as a taproot, is long, forked and plane yellow. As a biennial Conium maculatum produces a basal rosette in the first year, and then produces flowering stems during the second year. The stem of the plant is erect, smooth, slightly ridged, stout below, much branched above and hollow, and is bright green. (14)

(BChemical constituents: It contains piperidine alkaloids, flavonoids, vitamins, polyacetylenes, coumarins and volatile oils. There are eight known piperidine alkaloids include coiine, N-methycoiine, conhydrine, pseudoconhydrine, and gamma coiceine, that is predecessor of other hemlock alkaloids. Coniine is testified to beeight times more toxic than *y*-coniceine. (15)

(C) Taxonomy: Kingdom: plantae, Class: magnoliopsida, Family: apiaceace, Genus: conium, Species: Conium maculatum.

(DVvernacular names: Shaukaran, Bisbis bari, Cicuta funcho-selvagem, Du shen, Hemlock, Poison-hemlock, Carrot-fem, Fool's-parsely1, Spotted-hemlock. (16)

(E) Therapeutic uses: It is used as antiviral, anti-orchitis, neuro tonic, anti-cancer, and anti-spasmodic agents. (17,15)

3 AIM AND OBJECTIVES:

3.1 Aim:

The aim and objectives of the study is to carry out the ethanolic root extractions of Aconitum atrox and leafextraction of Conium maculatum.

3.2 Objectives:

(a) Collection and Authentication of plants.

(b)Plants extraction of Aconitum atrox and Conium maculatum.

(c)Phytochemical screening of plants.(d)Selection and grouping of animals.

(e)Screening for analgesics and anti-inflammatory using various screening techniques.

4. MATERIALS AND METHODS:

4.1 Collection and Authentication of Plant: The dried roots of Aconitum atrox family Ranunculaceae and driedleaves of conium maculatum Apiaceae will be obtained and authenticated by botanist; Dr. K Madhava Chetty, Assistant- professor, Department of Botany, Sri Venkateshwara University, Tirupati, A.P India.

4.2 Materials required: Porcelain jars, Beaker, Ethanol, Glass dishes, Foil wrap, Muslin cloth, standard drugs.

4.3 Preparation of plant extract: The pieces of roots and leaves on complete drying should be powdered and put in impermeable holders at room temperature. This powder will be macerated with ethanol for about 7 days, after which they will be separated. The extract obtained can be subjected for investigation of analgesic and anti- inflammatory actions.

4.4 Maceration: In this procedure, 500g/kg powder will be included in ethanol in the proportion 1:2. It will be agitated periodically for about 7 days consistently and kept at room temperature. The resulting filtrate will be assorted in 0.9% ordinary saline which will be used as a vehicle later for the tests to be performed.

4.5 Phytochemical Analysis:

Alkaloids: Now not many ml filtrate + 1-2 drops of Mayer's reagent (at the rims of check tube) a smooth white/ yellow precipitate might be seem, indicates presence of alkaloids.

Carbohydrates:2 ml filtrate + 2 drops of alcoholic a- naphthol + 1ml conc. H_2SO_4, a violet ring, is set up, whichdetermines the presence of starches.

Reducing sugars: 0.5 ml filtrate + zero. Five ml Benedict's reagent + Boiled for two min inexperienced/yellow/red color can be appeared, which shows presence of lowering sugars.

Glycosides:1 ml dil. H_2SO_4 + 0.2 ml conc. + bubbled for 15 min+ permitted cooling+ kill with 10% NaOH + 0.2 ml Fehling's solution A & B a brick crimson colored precipitate is formed, which famous presence of glycosides. **Flavonoids:** Aqueous solution infrequently any drops 10% ferric chloride association an inexperienced precipitate is fashioned, exhibit the presence of flavonoids.

Phenols: Extract aqueous solution+ no longer many drops 5% ferric chloride solution darkish inexperienced/bluish black tone well-known shows, indicates the presence of phenolic compounds.

Tannins:10ml of bromine water + 0.5gm plant extract Discoloration of bromine indicates the presence of tannins.**Saponins:** 0.5gm plant disposes of + 2ml water (overwhelmingly shaken) continual froth for 10 min. shows the presence of saponins.

Phytosterols/Steroids: Filtrate scarcely any drops of conc. H_2SO_4 (Shaken well and permitted to stand) crimson coloration (in the lower layer) demonstrates the presence of phytosterols.

Terpenoids: 2ml chloroform + 5ml plant cast off, (evaporated on water bathe) + 3ml conc. H_2SO_4 (boiled on water bathing) a gray-colored solution appears which suggests presence of terpenoids.

Coumarins: Plant extract + 10% NaOH+ Chloroform a yellow color well-known shows, which suggests thepresence of coumarins.

4.6 Experimental animals:

Male Albino Wistar rats weighing 150-200 gm will be used. The experimental animals will be maintained under standard laboratory conditions varying from 22-28°C, 12-hrs of light/dark cycle under controlled temperature. All the experimental animals should be familiarized with the laboratory surroundings for at least one-week earlier than the initiation of the experiment.

4.7 Experimental design:

Experimental animal design.

S.NO	GROUPS	TREATMENTS	DOSE AND ROUTE
1.	Group- 1	Normal Control	1ml- i.p
2.	Group- 2	Toxic Control	10 ml/ kg- i.p
3.	Group- 3	Standard Control	10 mg/kg- i.p
4.	Group- 4	AA Root Extract- d1	200 mg/kg-po
5.	Group- 5	AA Root Extract- d2	400 mg/kg-po
6.	Group- 6	CM Leaf Extract- d1	200 mg/kg-po
7.	Group- 7	CM Leaf Extract- d2	400 mg/kg-po
8.	Group- 8	AA+CM Leaf Extract- d1	200 mg/kg-po
9.	Group- 9	AA+CM Leaf Extract- d2	400mg/kg-po

***4.8 Collection of blood samples*:**

The blood samples are collected after 21 days of performing the experiment. The animals will be fasted for overnight and on 22nd day the blood is collected by retro orbital puncture with ether anesthesia using capillary tube in coagulant ampoule for estimation of serum level of different parameters such as Histamine, PGE2, Interleukins- 1,6,8 and cytokine TNF- alpha.

4.9 Histopathological Evaluation:

Animals will be fasted overnight and sacrificed with anesthesia, brain tissues samples to be kept in 10% formaldehyde solution and parameters will be assessed in the laboratory.

5.IN VIVO METHOD FOR ANALGESIC ACTIVITY

5.1 Hot plate method:

In the hot plate method was initially depicted by Woolfe and Mac Donald in the year 1944, which was further investigated by many others. In the hot plate method, heat is utilized as main stimulus to induce pain. Firstly, the rats were weighed, numbered and divided into 9groups of 6 critters each. Then each of them was subsequently instigated onto hot plate at temperature retaining 55°C for 15 sec succeeding instigation, Initially, the basal reaction time for paw licking or jumping is composed. Next Aspirin 20mg/kg i.p the standard drug, test drugs were administered and perpetually 15, 30 and 60 minutes later the basal reaction time was recorded.

There after the administration the reaction time shall be intensifying. The intensified time for the response recorded and compared accompanies standard and test drugs.

6.INVIVO METHOD FOR ANTI-INFLAMMATORY:

6.1 Carrageenan induced paw edema method:

In this method the anti- inflammatory activity of ethanol is carried out by Carrageenan induced paw edema method. The method utilizes albino rats weighing about 180-200gms and the critters were starved for overnight succeeding to treatment 30 minutes prior to carrageenan infusion. By following the exploratory design, the critters challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan into left hind paw at sub plantar region, ink marked at level of lateral malleolus and immersed in mercury.

Evalution: Paw volume of rat is measured by using plenthysmometer within 30 mins, 1hr, 2hr and 3hrs of interval. Then the raised in paw volume % is compared between the treated group and control group based on the averagevalue differences.

6. RESULTS:

7.1 Maceration: we got the results of ethanolic extract of plant 1 and 2 by maceration technique.

***7.2 Phytochemical screening results*:**

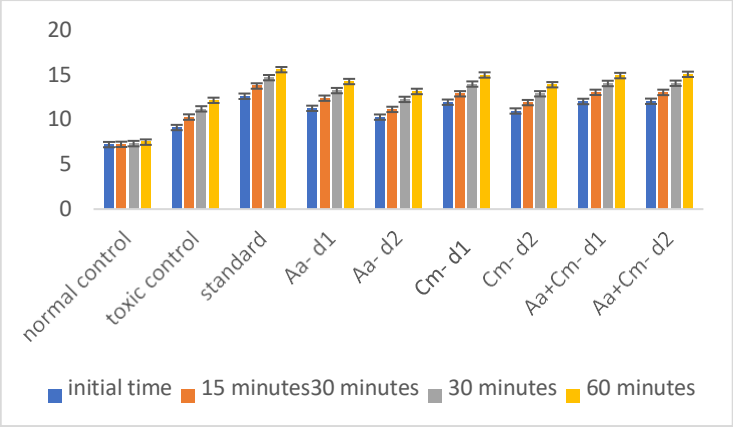
Types of chemical constituents	**Aconitum atrox root extract**	**Conium maculatum leaf extract**
Alkaloids	+	+
Glycosides	+	-
Carbohydrate	-	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Sterols	+	+
Phenols	+	+
Proteins	+	+
Triterpenoids	-	-

+ indicates presence; - indicates absences.

7.3 In vivo for analgesic activity:

Eddy's hot plate:

EDDY'S HOT PLATE METHOD:

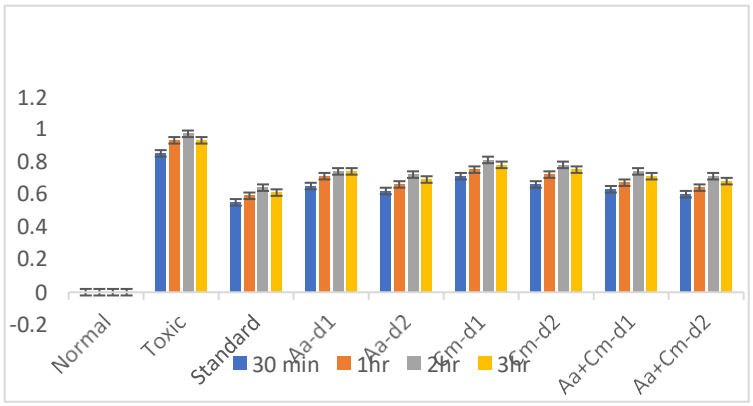


Graph-1: Effect of AAEE and CMEE on rats placed on hot plate

***7.4* In vivo for anti-inflammatory activity:**

Carrageenan induced paw edema method:

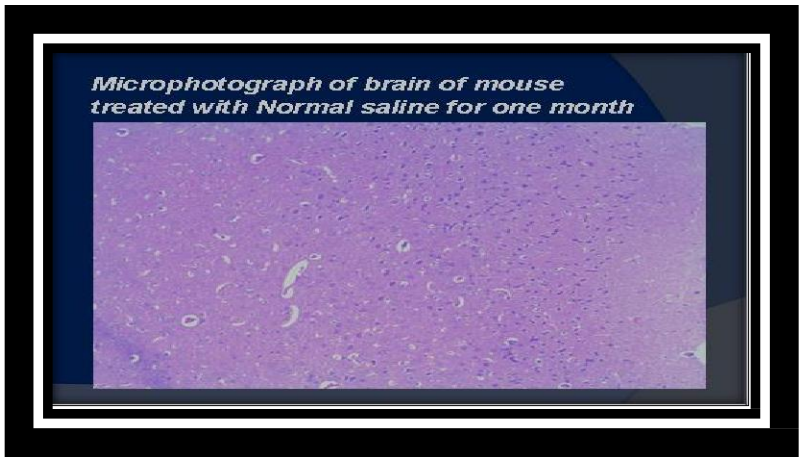
CARRAGEENAN INDUCED PAW EDEMA:



Graph-2: Effect of AAEE and CMEE on paw of carrageenan induced edema in rats

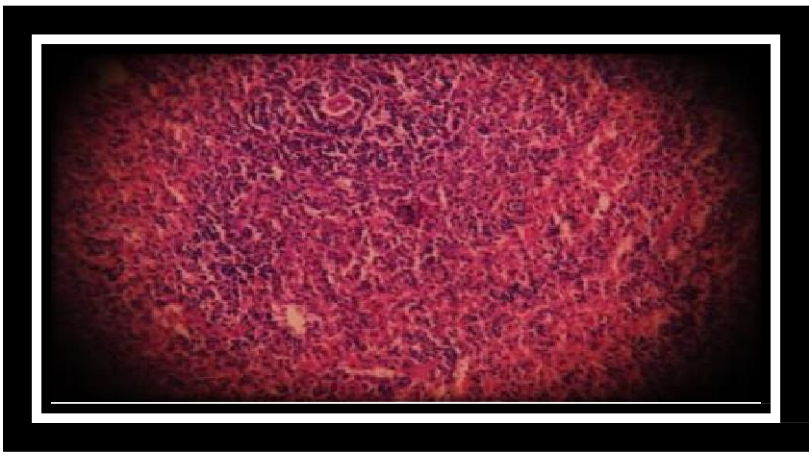
7.5 RESULTS OF HISTOPATHOLOGY:

GROUP-1:



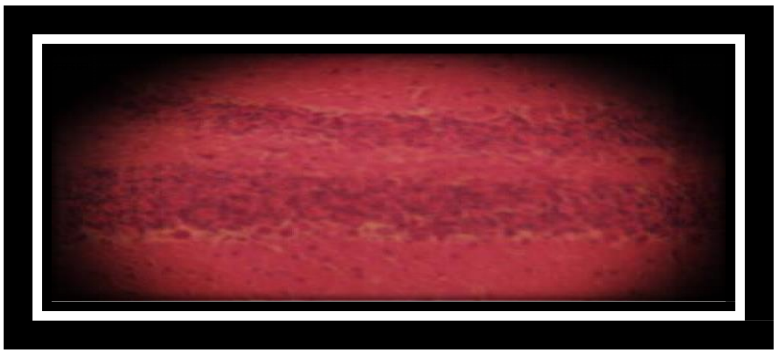
The figure shows normal brain tissue.

GROUP- 2:



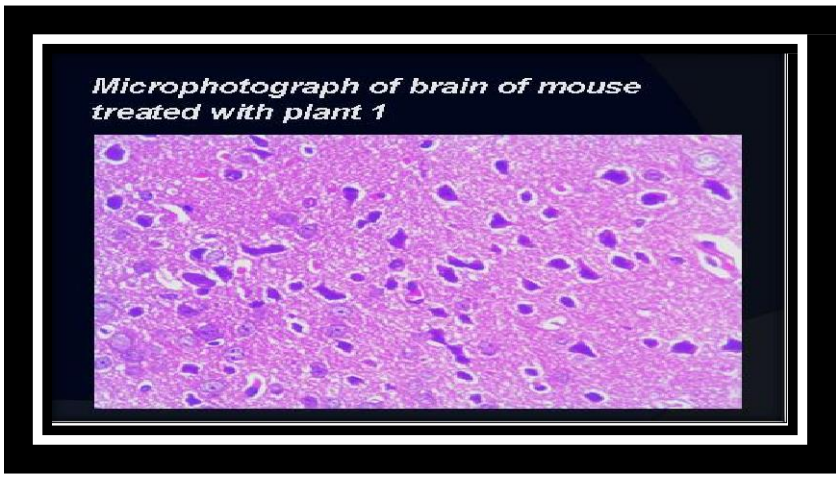
In the figure of group 2, the mice which are treated with toxic control demonstrate presence of no. of flame shaped CA3 hippocampal neurons and Deteriorated cells, basophilic appearance and Karyopyknosis.

GROUP- 3:



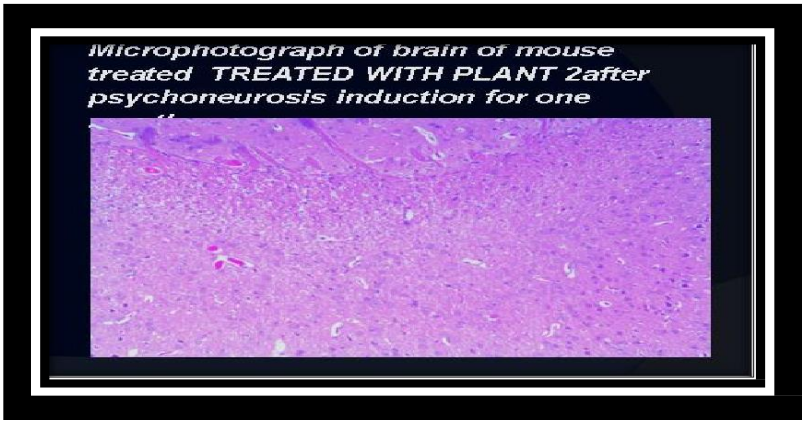
In the figure of group 3 mice, treated with standard drug by i. p route shows CA3 region with healthy cells of hippocampus and these group of mice had recovered their cells very closely to healthy cells.

GROUP – 4:



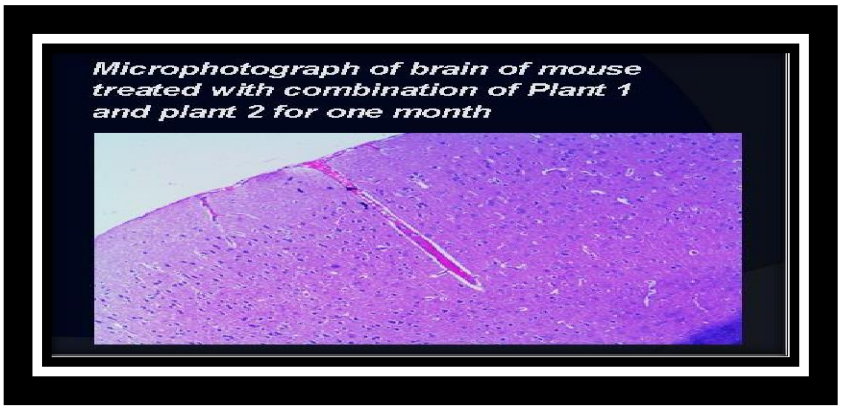
This group of animals demonstrate oedema of parenchyma and few neuronal degenerations.

GROUP- 5:



This group of animal exhibits neuronal degeneration and mild oedema.

GROUP- 6:



This group of animals demonstrate mild oedema and neurodegeneration.

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