JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Study of Analgesic Activity of Colocasia Esculenta (Linn.) Schott in Experimental Animals.

Miss. Shinde Shraddha B*[1], Prof. H. J. Pagar[2], Gondkar Shraddha[3]

Dr. Vitthalrao Vikhe Patil Foundation College of Pharmacy, Ahmednagar. Department of Pharmaceutical Pharmacology, Savitribai Phule Pune University, Pune, Maharashtra.

Email id: shindeshraddha203@gmail.com

ABSTRACT:

Colocasia esculenta Linn. Schott commonly called as Taro is cultivated in tropical and subtropical regions belongs to the family *Araceae.* The present study aimed to explore the analgesic activity of ethanolic leaves extract of *Colocasia esculenta Linn. Schott* using Hot plate, tail immersion, and acetic acid induced writhing model in mice. Intensive investigation on phytochemical constituents was done and found the presence of alkaloid, tannin and high amount of flavonoids. In this study, ethanolic leaves extract of *Colocasia esculenta Linn. Schott* at the dose of 100, 200 and 400 mg/kg was used and found significant (*p<0.05, **p<0.01 and ***p<0.001) analgesic activity which is highest at the dose of 400 mg/kg at reaction time 120 minutes in both hot plate and tail immersion model. The maximum inhibitory effect observed at the dose of 400 mg/kg in acetic acid induced writhing model. The present study concludes that this herbal medicine can be used as a painkiller.

KEYWORDS: *Colocasia esculenta Linn. Schott*, Analgesic, Acetic acid induced writhing, phytochemical constituents.

1. INTRODUCTION

Plants have been used as a potential source of medicine, due to an enormous diversity of bioactive compounds. Many of the plants used in the traditional medicine to alleviate the common ailments and to promote a healthy life [1, 2]. World Health Organization (WHO) mentioned that 80% of the world populations are dependent on the traditional medicine. India possesses well knowledge of the traditional medicine and practices it since ancient times [3]. In recent years, increased attention towards the use of herbal drugs has been observed throughout the world [4]. Studies have shown that opiates cause physical dependency, tolerance, and addiction while NSAIDs usually cause gastrointestinal disorders. Herbal therapy could be an interesting option for treatment of opioids dependence and withdrawal. There is enormous development in pain therapies; the medicinal community still needs safe, effective and potent

analgesic drugs for the treatment of different painful conditions [5]. *Colocasia esculenta (Linn.) Schott.* has been traditionally used for relieving pain and injuries. So, there were no claims in the modern literature about analgesic activity of *Colocasia esculenta (Linn.) Schott.* Therefore, the present study was planned to evaluate the traditional claims of analgesic activity of *Colocasia esculenta (Linn.) Schott.* in experimental animals. It was found that flavonoids, alkaloids and tannins phytochemical were present.

Analgesic: [6]

A drug that selectively relieves pain by acting in the central nervous system or on peripheral pain mechanism, without significantly altering consciousness.

Analgesia is an ill-defined, unpleasant sensation, usually evoked by an external or internal noxious stimulus.

Pain is a warning signal, primarily protective in nature, but causes discomfort and suffering; may even be unbearable and incapacitating. Excessive pain may produce other effect such as sinking sensation, apprehension, sweating, nausea, palpitation, rise or fall in blood pressure, tachypnoea.

Analgesics relieve pain as a symptom, without affecting its cause. They are used when noxious stimuli cannot be removed.

It is divided into two groups narcotics and non-narcotics.

Table no.1. : Comparative study of narcotic and non-narcotic analgesics.

Narcotic analgesics	Non- Narcotic analgesics
Act centrally	Act peripherally
Cause addiction	Do not cause addiction
Produce CNS depression	Do not produce CNS depression
No gastric irritation	Produce gastric irritation
No anti-inflammatory effect	Show anti-inflammatory effect
e.g. morphine, tramadol, pethidine	e.g. Diclofenac, ibuprofen, aspirin

Mode of action of analgesics: [7]

- Analgesic drugs act in various ways on the peripheral and central nervous systems.
- Opioids produce analgesia by binding to specific G-protein coupled receptors in brain and spinal cord.
- NSAIDs inhibit the activity of both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) and thereby the synthesis of prostaglandins and thromboxanes.
- Inhibition of COX-2 leads to the anti-inflammatory, analgesic and anti-pyretic effects.

Recent advances: [7]

Some novel and investigational analgesics include sub type-selective voltage gated sodium channel blockers such as funapide and raxatrigine, as well as multimodal agents such as ralfinamide.

Herbal drugs used: [8]

Numerous medicinal plants and their derived phytochemical were evaluated for their analgesic activity.

- The *Salix alba* (willow bark) contains a bioactive compound salicin, which hydrolyses into salicylic acid and act as pain killer by inhibiting the COX-1 and COX-2.
- *Pistacia integerrima* contain pistagremic acid, inhibits COX-2 enzyme.

- *Papaver somniferum* which contains a potent narcotic alkaloid called morphine binds to opioids receptor and leads to analgesic.
- *Salvia divinorum* contains Salv1inorin A acts as κ-opioid receptor agonist.
- *Dalea purpurea* contains Pawhuskin A acts as opioids receptor antagonist.

Colocasia esculenta:

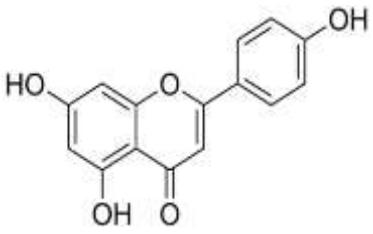
Colocasia esculenta (Linn.) Schott is a tropical plant grown primarily for its edible corms, a root vegetable most commonly known as Taro [9]. It is major root crop belongs to the family *Araceae*, sub family *Aroideae*.

Figure no. 1: *Colocasia esculenta (Linn.) Schott*

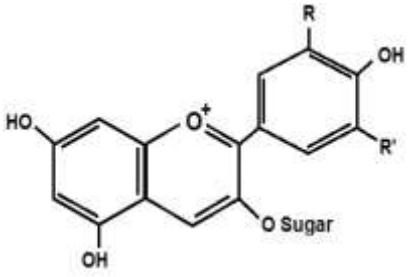
Phytochemistry: [10]

Mainly leaves contain calcium oxalate, fibers, minerals (calcium phosphorous, etc.), starch, vitamin A, B, C etc. Phytochemically, these also contain flavones, apigenin, luteolin and anthocyanins. The isolated flavonoids contain vicenin-2, iso-vitexin, iso-vitexin 3'-O-glucoside, vitexin X''-O-glucoside, iso-orientin, orientin, orientin 7-O-glucoside, luteolin 7-O-glucoside. It also contains tarin, alkaloid, saponin, tannin, polyphenols, polysaccharide (arabinogalactan).

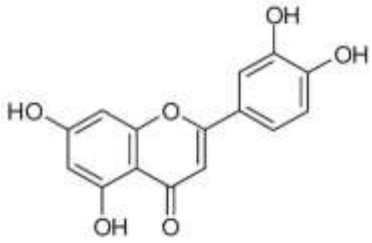
Colocasia esculenta Linn. Schott tubers contain globulins accounting for 80% of the total tuber proteins. Taro corms have been reported to have 70-80% starch with small granules. The high level of carbohydrate content observed in raw taro, taro powder and total amino acids recorded in the tubers are in the range of 1380-2397 mg/100g.



Apigenin



Anthocyanin



Luteolin

Figure no.2: Structure of chemical constituents of *Colocasia esculenta Linn. Schott*

2. MATERIALS AND METHODS:

Equipments required:

- Soxhlet extractor, Hot plate model, Tail immersion model.
- Syringe, Oral feeding tube, Glassware.

These were obtained from Dr. Vitthalrao Vikhe Patil Foundation's College of Pharmacy, Ahmednagar, Maharashtra.

Animals:

Albino wistar rats weighing 120-200 grams were received from animal house of Dr. Vitthalrao Vikhe Patil Foundation's College of Pharmacy, Ahmednagar, Maharashtra. The study was conducted after obtaining approval from Committee for the Purpose of Control and Supervision on Animals (CPCSEA) and Institutional Animal Ethics Committee (IAEC), proposal number (1670/PO/ReBiBt/S/12/CPCSEA).

On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2˚C and relative humidity of 30-70%. A 12:12 light:dark cycle was followed. All animals were allowed to free access to water and bed with standard commercial pelleted chow.

All the experimental procedures and porotocols used in this study were reviewed by Institutional Animal Ethics Committee (1670/PO/ReBiBt/S/12/CPCSEA).

Chemicals required:

- 70% ethanol, Dragendroff's reagent, Mayer's reagent, Sulphuric acid, Lead acetate solution, Sodium hydroxide, Bromine water.
- **Drugs and solutions:** Saline solution, Pentazocine, Diclofenac sodium, 1% Acetic acid solution.

The chemicals and drugs were issued from the laboratory of Dr. Vitthalrao Vikhe Patil Foundation's College of Pharmacy, Ahmednagar, Maharashtra.

- **Reference and control drugs:** [13, 14]

Standard drug: Pentazocine, Diclofenac sodium.
Control: DMSO + Water, 1:1

Collection of plant parts and authentication:

Plant of *Colocasia esculenta Linn. Schott* leaves was collected from the village of Loni in Ahmednagar, Maharashtra, India in the month of February 2021 in the amount enough for all the experiment and plant material was authenticated at Department of Botany and Research Centre, Padmashree Vikhe Patil College of Arts, Science and Commerce, Pravaranagar. The leaves were then washed under running tap water and shade dried (25˚C ± 5% Relative humidity) for 8 days. The shade dried leaves was pulverized with a dry grinder to get the coarse powder. The powder was stored in air tight container for further use.

Drying and pulverization of plant material:

After collection and authentication the leaves were washed to remove the dust particles and allowed to dry in a shade for complete drying. Then the dried leaves without moisture were powdered in a mixer grinder.

Preparation of the plant extract: [11]

The coarse powder was packed tightly in the soxhlet apparatus and extracted with ethanol for 8 hours and temperature was maintained at 60˚C throughout the extraction process. The extract was then collected and solvent was evaporated under vacuum. The resulting ethanolic extract was stored at 4˚C and subjected to further phytochemical study.

Phytochemical analysis: [12]

The ethanolic extract of *Colocasia esculenta Linn. Schott* leaves were subjected to qualitative phytochemical tests for different constituents such as alkaloids, flavonoids, tannins and saponins.

1. Test for alkaloids:

Dilute the alcoholic extract, shake well and filter**.**

- **Dragendroff's test:**

To 2-3 ml filtrate, add few drops of Dragendroff's reagent and orange brown precipitate were observed as result.

- **Mayer's test:**

To 2-3ml filtrate, add few drops of Mayer's reagent which gives precipitate as result.

2. Test for flavonoids:

- **Sulphuric acid test:**

To the extract sulphuric acid (60% or 80%) was added which gives deep yellow solution indicates the presence of flavonols.

- **Lead acetate:**

To the extract add lead acetate solution which gives yellow colour precipitate

- **Acid base test:**

Addition of increasing amount of sodium hydroxide to the residue shows coloration which decolorizes after addition of acid

3. Test for tannins:

- **Bromine water:**

2-3 ml extract add bromine water which shows discoloration of bromine water.

- **Dilute HNO_3:**

2-3 ml extract, dilute sulphuric acid was added which shows reddish to yellow colour.

4. Test for saponin:

- **Foam test:**

Shake the drug extract vigorously with water. Foam will be observed.

Statistical analysis:

The values were expressed as mean ± SEM (n=6). The statistical significance was assessed using one-way analysis (ANOVA) followed by Dunnett's test and *p<0.05, **p<0.01 and ***p<0.001 were considered to be statistically significant.

3. PHARMACOLOGICAL STUDIES:

1) Hot plate method in rat: [14, 15, 16,]

Principle:

In this method heat is used as a source of pain. Animals are individually placed on hot plate maintained at constant temperature (55° C) and the reaction of animals, such as paw licking or jump response is taken as the end point. Analgesics increase the reaction time.

Procedure:

This method is used for the assessment of centrally acting analgesic drugs. The central analgesic drug Pentazocine is used as standard. In this experiment, five groups (n=6) of wistar rats (120-200 g) were used.

- Group-I: DMSO + water (1:1) served as normal control,
- Group-II: Pentazocine (3mg/kg i.p.) served as standard
- Group-III: Ethanolic extract of *Colocasia esculenta (Linn.) Schott.* 100mg/kg p.o served as test.
- Group-IV: Ethanolic extract of *Colocasia esculenta (Linn.) Schott.* 200mg/kg p.o served as test.
- Group-V: Ethanolic extract of *Colocasia esculenta (Linn.) Schott.* 400 mg/kg p.o served as test.

Hot plate consists of an electrically heated surface which is maintained at room temperature for 15 minutes. Food was withdrawn on the preceding night of the experiment. On the day of experiment the animals were individually placed on Eddy's Hot Plate at 55˚C and the time until either licking or jumping occurs is recorded by using stop-watch. Latency to exhibit nociceptive responses was determined at 30, 60, 90 and 120 minutes after administration of the drugs or vehicle.

2) Tail immersion test: [14, 15, 17]

Principle:

The use of immersion of the tail is apparently a variant of the tail flick model. The most obvious difference is that the area of stimulation is far greater. Immersion of an animal's tail in hot water provokes an abrupt movement of the tail and sometimes the recoiling of the whole body. Again, here it is the reaction time that is measured.

Procedure:

This method was used to evaluate the centrally acting analgesic effect of drug. The wistar rats were divided into 5 groups (n=6).

- Group-I: DMSO + water (1:1) served as normal control,
- Group-II: Pentazocine (3mg/kg i.p.) served as standard
- Group-III: Ethanolic extract of *Colocasia esculenta (Linn.) Schott.* 100mg/kg p.o served as test.
- Group-IV: Ethanolic extract of *Colocasia esculenta (Linn.) Schott.* 200mg/kg p.o served as test.
- Group-V: Ethanolic extract of *Colocasia esculenta (Linn.) Schott.* 400 mg/kg p.o served as test.

They were placed into individual restraining cages leaving the tail hanging out freely. The lower 5cm portion of the tail was marked and this part of tail was immersed in a water bath containing water at a

temperature of 55 ± 0.5˚C. Within few seconds the rat reacts by withdrawing the tail from the hot water. The reaction time was noted on a stop-watch. After each determination the tail is carefully dried. The reaction time of the groups was taken at 0, 30, 60, 90 and 120 minutes. The cut off time of the immersion was 15 seconds. The reaction time was measured.

3) Acetic acid induced writhing: [14, 16]

Principle:

Painful reactions in animals may be produced by chemical also. Intraperitoneal injection of phenylquinone, bradykinin or acetic acid produces pain reaction which is characterized as a writhing response. Abdominal constriction, turning of trunk (twist) and extension of hind legs (stretching) responses by the animal are taken as reaction to chemically induced pain. Analgesics, both narcotic and non-narcotic type, inhibit writhing response.

Procedure:

This method is used for the assessment of peripheral analgesic effect of drug. Five groups (n=6) of wistar rats was fasted overnight prior to start the experiment with free access to water.

- Group-I: DMSO + water (1:1) served as normal control,
- Group-II: Diclofenac sodium (10mg/kg) served as standard
- Group-III: Ethanolic extract of *Colocasia esculenta (Linn.) Schott.* 100mg/kg p.o served as test.
- Group-IV: Ethanolic extract of *Colocasia esculenta (Linn.) Schott.* 200mg/kg p.o served as test.
- Group-V: Ethanolic extract of *Colocasia esculenta (Linn.) Schott.* 400 mg/kg p.o served as test.

After 30 minutes of treatment, the mice were injected intra-peritoneally with 0.1 ml of 1% acetic acid solution to induce the characteristic writhing. The mice were then placed in an observation box and the numbers of writhing were counted in a 10 minutes period. The response of the extract and Diclofenac sodium treated groups was compared with those of animals in the control groups.

4. RESULT AND DISCUSSION:

Results:

1) Phytochemical analysis of ethanolic extract of *Colocasia esculenta (Linn.) Schott.*

Table no.2: Phytochemical analysis of ethanolic extract of *Colocasia esculenta (Linn.) Schott.* leaves:

Phytochemical	Inference
Alkaloid	+
Flavonoid	++
Tannin	+
Saponin	-

++: high content

+: moderate

- : negative.

2) Analgesic activity:

- **Hot plate method:**

Table no.3: Analgesic effect of ethanolic extract of *Colocasia esculenta (Linn.) Schott.* on hot plate model.

Group	Paw licking or jumping response in seconds			
	30 min	60 min	90 min	120 min
Group-I Control	2.3±0.20	2.6±0.15	2.8±0.10	2.9±0.10
Group-II Pentazocine (3mg/kg)	2.6±0.16**	6.7±0.15**	9.6±0.64*	9.7±0.15**
Group-III (100mg/kg)	2.4±0.15**	2.5±0.07**	2.7±0.01**	2.8±0.01**
Group-IV (200mg/kg)	2.6±0.17*	4.8±0.05**	7.3±0.05***	7.4±0.24**
Group-V (400mg/kg)	2.7±0.15*	6.4±0.05**	8.9±0.24***	9.3±0.05***

Values were mean ± SEM, (n=6), ns non- significant, *p<0.05, **p<0.01, ***p<0.001. when compared with control. Data was analyzed by using One-way ANOVA followed by Dunnett's "t" test.

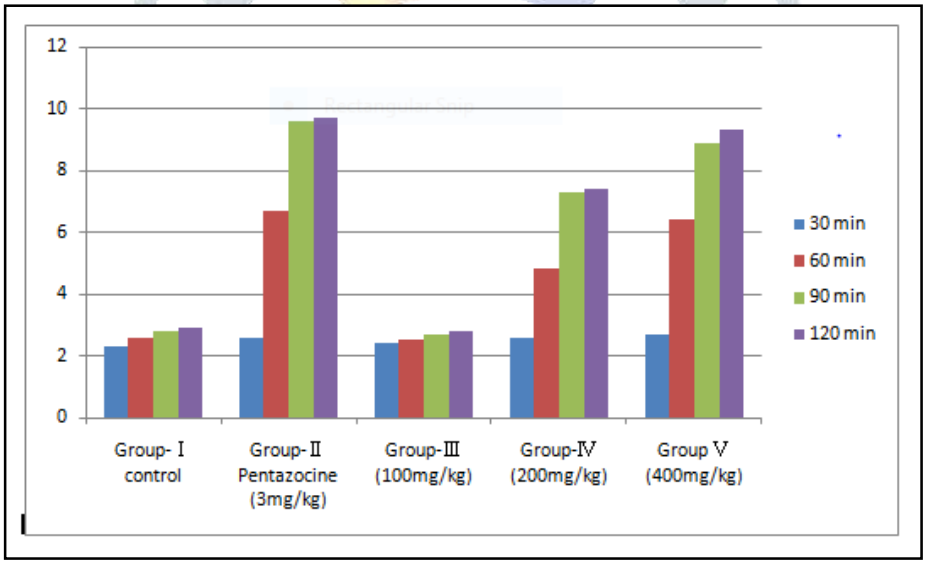


Figure no.3: Graphical representation of Hot plate analysis

- **Tail immersion model:**

Table no. 4: Analgesic effect of ethanolic extract of *Colocasia esculenta (Linn.) Schott.* on Tail immersion model.

Group	Tail immersion reaction in seconds				
	0 min	30 min	60 min	90 min	120 min
Group-I Control	1.2±0.05	1.3±0.05	1.5±0.01	1.6±0.04	1.8±0.02
Group-II Pentazocine (3mg/kg)	1.6±0.02**	2.5±0.02**	4.5±0.05***	5.7±0.01***	5.9±0.05**
Group-III (100mg/kg)	1.2±0.01*	1.5±0.02**	1.9±0.04**	2.1±0.03**	2.2±0.01**
Group-IV (200mg/kg)	1.4±0.04*	1.8±0.06*	2.5±0.08***	3.2±0.02***	3.6±0.05***
Group-V (400mg/kg)	1.5±0.03**	2.2±0.01**	2.7±0.02***	3.8±0.01***	4.3±0.03***

Values were mean ± SEM, (n=6), ns non- significant, *p<0.05, **p<0.01, ***p<0.001. when compared with control. Data was analyzed by using One-way ANOVA followed by Dunnett's "t" test.

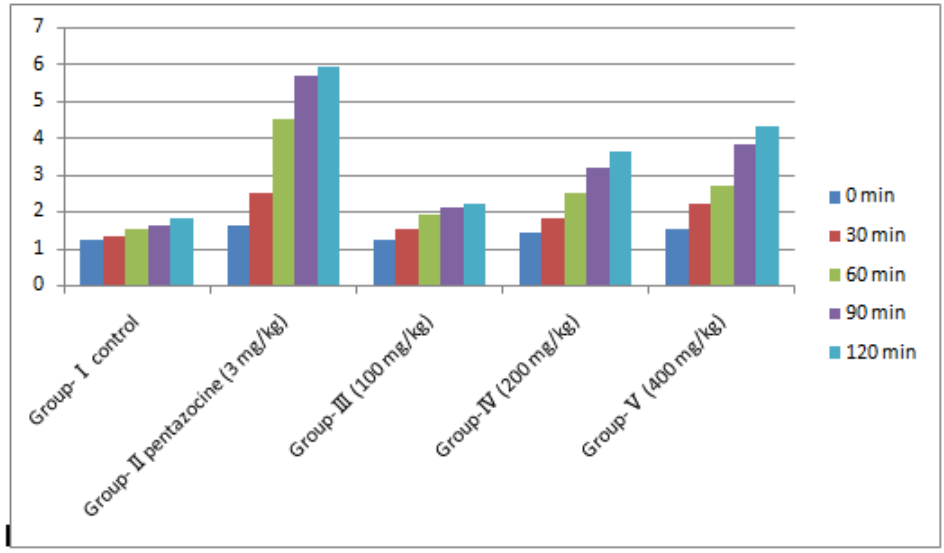


Figure no.4: Graphical representation of Tail immersion analysis.

Acetic acid induced writhing:

Table no. 5: Analgesic effect of ethanolic extract of *Colocasia esculenta (Linn.) Schott.* on acetic acid induced writhing in rat.

Group	Mean number of writhes	% Inhibition
Group-I Control	5.8 ±0.51	-
Group-II Diclofenac sodium (10mg/kg)	4.0 ± 0.49	31.03 %
Group-III (100mg/kg)	4.6 ± 0.51	20.685
Group-IV (200mg/kg)	4.4 ± 0.37	24.13%
Group-V (400mg/kg)	4.1 ± 0.40	29.31%

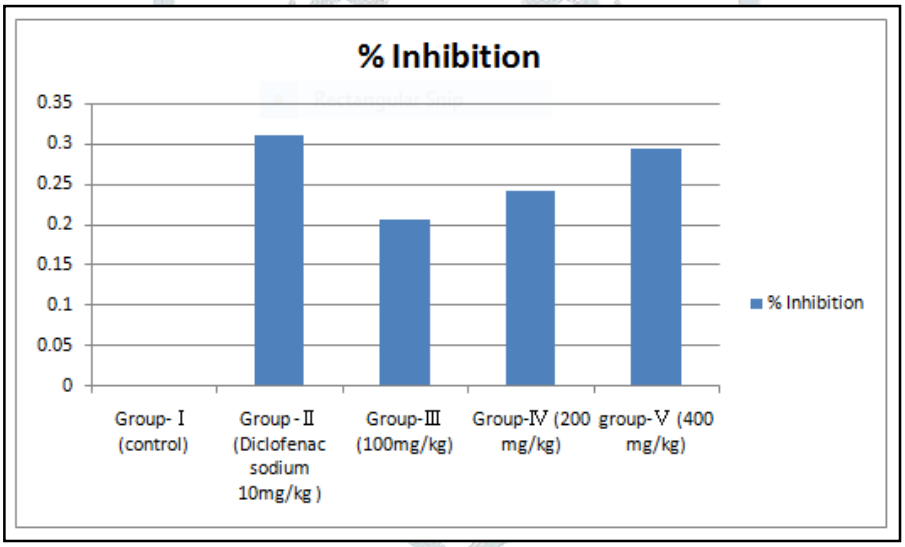


Figure no.5: Graphical representation of percentage inhibition of Acetic acid induced writhing in rat.

Discussion:

Analgesics are the agents which selectively relieve pain by acting in the CNS or peripheral pain mechanism without significantly altering consciousness.

Three analgesic models were used to evaluate the analgesic activity i.e. Hot plate model, Tail immersion model and Acetic acid induced writhing model. It involves the reaction of animals to painful stimuli which may be thermal, mechanical or chemical.

Phytochemical studies:

From the qualitative phytochemical analysis of ethanolic extract of *Colocasia esculenta (Linn.) Schott.* it was found that it contains alkaloids, tannins and high amount of flavonoids.

Hot plate method:

In this method heat is used as a source of pain. Animals were individually placed on hot plate maintained at constant temperature (55° C) and the reaction of animals, such as paw licking or jump response is taken as the end point. Analgesics increase the reaction time.

The analgesic activity of ethanolic leaves extract of *Colocasia esculenta (Linn.) Schott.* was assessed at dose of 100, 200 and 400 mg/kg. Analgesic activity was compared with standard drug pentazocine (3mg/kg i.p.). Among all three doses 400 mg/kg showed maximum analgesic activity at reaction time 120 min (9.3 ± 0.05) which is slightly lower than the standard drug pentazocine (9.7 ± 0.15), it prolonged the reaction time of animals with relatively extended duration of stimulation, confirming centrally active drug. In the present study, all ethanolic extracts showed significant (*p< 0.05, **p<0.01 and ***p<0.001) analgesic activity.

Tail immersion method:

The use of immersion of the tail is apparently a variant of the tail flick model. The most obvious difference is that the area of stimulation is far greater. Immersion of an animal's tail in hot water provokes an abrupt movement of the tail and sometimes the recoiling of the whole body. Again, here it is the reaction time that is measured.

The analgesic activity of ethanolic leaves extract of *Colocasia esculenta (Linn.) Schott.* was assessed at dose of 100, 200 and 400 mg/kg. There was a significant (*p<0.05, **p<0.01 and ***p<0.001) reduction of pain full sensation due to tail immersion in warm water. Among all three doses, maximum analgesic activity observed at the dose of 400 mg/kg at 120 min (4.3±0.03) which is slightly lower than the standard drug pentazocine (5.9± 0.05).

Acetic acid induced writhing:

Painful reactions in animals may be produced by chemical also. Intraperitoneal injection of phenylquinone, bradykinin or acetic acid produces pain reaction which is characterized as a writhing response. Abdominal constriction, turning of trunk (twist) and extension of hind legs (stretching) responses by the animal are taken as reaction to chemically induced pain. Analgesics, both narcotic and non-narcotic type, inhibit writhing response.

The ethanolic leaves extract of *Colocasia esculenta (Linn.) Schott.* showed reduction in writhing at the dose of 100, 200 and 400 mg/kg as (4.6 ±0.51, 4.4±0.37 and 4.1±0.40) respectively. Injection of acetic acid injected into control mice produced 5.8± 0.51 writhes. Among all three doses maximum inhibitory effect was observed at 400 mg/kg (29.31%) which is slightly lower than the standard drug Diclofenac sodium (31.03%).

5. CONCLUSION:

The present thesis entitled "Study of analgesic activity of Colocasia *esculenta (Linn.) Schott* in experimental animal" deals with the exploration of phytochemical and pharmacological screening of the selected Indian medicinal plant *Colocasia esculenta (Linn.) Schott* belongs to the family Araceae. It contains alkaloid, tannin and high amount of flavonoids. It was reported that the flavonoids frequently found in plants possess analgesic activity. Ethanolic leaves extract of *Colocasia esculenta (Linn.) Schott* possess several pharmaceutical and pharmacological properties. The study concludes that this herbal medicine can be used as an alternative therapy for the treatment of minor to moderate types of pain and act as painkiller. Further study will enable us to understand the mechanism of action of the above mentioned activity.

6. Future scope:

- Herbal medicines are increasingly used to treat all kinds of disorders from mild cases to severe diseases in developed and developing countries of the world. Many of the modern medicines are produced indirectly from medicinal plants.
- It is often noted that over 25% of all drugs prescribed today come from plants. Healthcare professionals and the public are expressing concern about the safety, efficacy, quality, availability, preservation and further development problems of these herbal products.
- Phytochemical and pharmacological researches are going on the herbal medicines and efforts are being made to isolate and identify their active chemical constituents and to substantiate the claims of the safety and efficacy. Traditional medicines (TM) remain widespread in developing countries and Complementary and Alternative Medicine (CAM) is increasing rapidly in developed countries.
- Similarly from the research it is proved that *Colocasia esculenta (Linn.) Schott* is herbal plant which can be used as an analgesic to treat various kinds of pain. It acts centrally as well as peripherally to inhibit the sensation of pain which was tested on animal model.
- Further appropriate pharmaceutical formulation need to be developed and clinical trials is need to be done to check its safety and efficacy so that this herbal medicine can overcome the problems faced by using synthetic medicines. It can be used as an alternative to narcotic and non-narcotic drugs that produce many side effects and addiction.
- Further, proper knowledge about herbal products is important as it may cause side effects or adverse effects.
- Additionally, use of adulterants and inappropriate formulation must be controlled which produce low quality product that could be harmful and even more dangerous.
- At last it is concluded that herbal medicine hold good future and they, may one day emerge as better alternative to synthetic chemical based allopathic drugs or may even replace them.

7. REFERENCE:

1. Patel J.K. and Patel P. Y., "Botanical therapeutics: discovery, development and manufacture-prospects and constraints," Journal of Natural Remedies, 2007; 7(1); 19-30.

2. Yadav M.O. and Parle M. I., "A simple laboratory model for inducing and measuring pain in small experimental animals," International Journal of Pharmacy and Pharmaceutical Science; 2016 8(7): 156-162.

3. Gupta S.K., "Drug Screening Methods," 2nd edition, Jaypee Brothers Medicinal Publishers, New Delhi, 2009: 480-498.

4. Kumar S., Vandana U.K., Agrwal D. and Hansa J., "Analgesic, Anti-inflammatory and Anti-pyretic effects of Azadirachta indica (neem) leaf extract in albino rats," International Journal and Science Research, 2015: 4(7), 13-21.

5. J.M.Pires, F. R. Mendes, G. Negri, J. M. Duarte-Almeida and E. A. Karlini, "Antinociceptive peripheral effect of Achillea millefolium L. and Artemisia vulgaris L. : both plants known popularly by brand names of analgesic drugs," Phytotherapy Research,2009, vol. 23, no. 2, pp. 212-219.

6. Essentials of Medical Pharmacology, K. D. Tripathi, Jaypee brothers medical publishers, 6th edition, 184-201, 453-468.

7. Lynn Marks, "What is an Analgesic?" medically reviewed by Robert Jasmer, MD, Everyday Health Newsletters, Nov. 25, 2015.

8. Abdur Rauf, Noor Jahan, Zarka Ahmad, Mohammad S. Mubarak. "Analgesic potential of extracts and derived natural products from medicinal plants". Chapter 17, page no. 339-351.

9. Goncalves RF, Silva AM, Valentao P, Ferreres F, Gil-lzquierdo A et al. "Influence of taro (Colocasia esculents L. Schott) growth conditions on the phenolic composition and biological properties". Science direct 2013, 141(4) : 3480-3485.

10. Krishnapriya TV, Suganthi A. "Biochemical and phytochemical analysis of Colocasia esculenta (L.) Schott tubers". International Journal of Research in Pharmacy and Pharmaceutical Science, 2017; 2(3): 21-25.

11. Deshmukh T.A., Kumawat N.S., Chaudhari S.P., Wani N.S., Patil V. R. " Antidiabetic activity of ethanol extract of Colocasia esculenta leaves in alloxan induced diabetic rats". International Journal of PharmTech Research. 2010, Vol. 2, No. 2, 1246-1249.

12. Pharmacognosy techniques and experiments, Dr. K. R. Khandelwal and Dr. Vrunda Sethi, Nirali prakashan, 27th edition, 25.4-25.6.

13. Mohamad Ali Hijazi, Ahmed El-Mallah, Maha Aboul-Ela, Abdalla Ellakany. "Evaluation of Analgesic activity of Papaver libanoticum extract in mice: Involvement of opioids receptors". Hindawi evidence based complementary and alternative medicine, 2017, 1-13.

14. Geeta Devi Sahu, Vishal Shrivarstava, Parul Senger, Jay Prakash Rai. "Evaluate analgesic and anti-inflammatory effect of ethanolic leaves extract of Solanum virginianum (L.)". Indian Journal of Applied Research, June 2020; Vol. 10, Issue 6, 8-13.

15. Drug Discovery and Evaluation: Pharmacological assays, Hans Gerhard Vogel, 3rd edition, Vol. 2, 1013- 1016.

16. Handbook of Experimental Pharmacology, S. K. Kulkarni, 4th edition, reprint 2019, Vallabh Prakashan, 139-142.

17. Parle Milind and Yadav Monu, "Laboratory models for screening analgesics". International Research Journal of Pharmacy. 2013, 4(1), ISSN 2230-8407, 15-19.