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**Research Article**

# ANALGESIC ACTIVITY OF DIFFERENT SOLVENT EXTRACT OF *OPERCULINA TURPETHUM* BY USING SWISS ALBINO MICE

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**ABSTRACT**

Operculina turpethum is a commonly used plant in the treatment of skin allergic conditions, fever, constipation, anorexia, ulcers, wounds etc. This plant is used in tribal areas as an analgesic, anti inflammatory etc. The present study is an attempt to explore the analgesic activity of chloroform and petroleum ether extracts of Operculina turpethum plant by acetic acid induced writhing method and tail immersion method in Swiss albino mice. In this study Diclofenac sodium is used as a standard drug and normal saline used as a vehicle control. The results of this study revealed that the plant extracts exhibit significant activity almost equal to that of standard drug.

**Keywords:** Operculina turpethum, writhing method, tail immersion method, Diclofenac sodium.

## INTRODUCTION

Operculina turpethum it is also called trivrit is an important herb and is a large stout perennial twinner with milky juice and fleshy branched roots .[1] It is used in ayurvedic system of medicine since ages. Root bark, root stem and leaves of this herb have high medicinal value [2]. The plant have high medicinal value. It is one of the plants mentioned in the literature having claims of activity against liver disorders [3]. It also has anthelmintic expectorant, antipyretic, analgesic, anti-inflammatory and purgative properties. It contains a wide variety of phyto constituents, which are useful in treatment of different ailments and includes glycosidic resin, coumarins, beta-sitosterol, and essential oils [4].

*Operculina turpethum* is a perennial herb with milky juice and its root is incorporated in the Avipattikara churna (An Ayurvedic preparation used for the treatment of hyperacidity, gastric ulcer and related gastrointestinal disturbances) [5].

The root bark of Trivrit is rich in turpethum resin consisting of 10% 'turpethin' which is a glycoside analogue of Jalapine and Convolvulin and is insoluble in ether, benzene, carbon sulphide and essential oils. Under the action of alkaline bases, turpethin is transformed into turpethic acid, while it gets converted into turpetholic acid, Glucose and fructose in presence of hydrochloric acid. Trivit also contains Turpethinic acids- A, B, C, D, & E,[6] some ether soluble resin, volatile oil, albumin, starch, lignin salts, ferric oxide, Scopoleptin, Betulin, lupiol & beta- sitosterol Turpethin is mainly responsible for purgative action of Trivit and is an excellent relatively safer substitute for jalap[7].

In Ayurveda, root of Trivit is used internally to treat fevers, anorexia, edema, anemia, ascites constipation, hepato-splenomegaly, hepatitis, intoxication, abdominal tumors, ulcers, wounds, worm infestation, pruritis and other skin disorders.[8] Root is also administered to treat obesity, hemorrhoids, cough, asthma,[9] dyspepsia, flatulence, paralysis, gout, rheumatism, melancholia, scorpion sting, and snake bites. The paste of root powder of Trivrit is used topically to treat vitiligo & other skin disorders, alopecia, cervical lymphadenitis, hemorrhoids, fistulas, ulcers, & chancres.[10] Oil extracted from the root bark of Trivrit is used in skin diseases of a scaly nature.[11] A processed ghee with Trivrit or fresh juice of Trivrit leaves is dropped into the eyes to treat diseases like corneal opacity or ulcer and conjunctivitis. Root powder of Trivrit mixed with ghee and honey is also used to treat hematemesis, tuberculosis & herpes.

## MATERIALS AND METHODS

### Plant collection

The whole plant of *Operculina turpethum* plant was procured from chirala and the plant was identified and authentified in department of botany, govt. Degree College, chirala. The plant material was thoroughly washed, dried and powdered, then used for extraction.

### Preparation of extract

The whole plant material was extracted with chloroform and petroleum ether using soxhlet extraction apparatus for six to seven hours at a temperature not exceeding the boiling point of the solvent used. The extracted solvent was subjected to distillation to separate the solvent and crude residue. Then the obtained residue is redissolved in dimethyl sulfoxide to get different concentrations of crude extract and stored in sterile brown bottles in a freezer at $20^0$ C until bioassayed.

### Experimental animals

The experiment of analgesic activity was conducted (as per CPCSEA guide lines) on S*wiss albino* mice of both sexes, aged 4-5 weeks, weighing about 20 – 25 g. before initiating the experiment, the mice were acclimatized for few days under standard environmental conditions ( 12 hours dark / 12 hours light cycle ; temperature 20-$22^0$C).

### Phytochemical evaluation

The crude extract was subjected to standard Phytochemical screening tests for various constituents like alkaloids, glycosides, carbohydrates, reducing sugars, proteins, steroids, flavonoids, tannins, phenolic compounds and volatile oils by using standard methods. (The results were tabulated in table.1).

### Experimental section

The analgesic activity was performed by two methods.

### Acetic acid induced writhing method

In this method, Animals were divided into four groups, four animals in each. Group I served as vehicle control receiving normal saline. Group II served as positive control Diclofenac sodium (10 mg/kg, i.p) was given. Groups III & IV received chloroform and petroleum ether extracts of *Operculina turpethum* (125, 250, 500 and 1000 mg/kg). Analgesic activity of *Operculina turpethum* (125, 250, 500 and 1000 mg/kg) was assessed by counting the number of writhes induced by 0.6% acetic acid (10 ml/kg, i.p.). Number of writhes per animal was counted in the following 20 min. Diclofenac sodium (10 mg/kg, i.p) was used as a positive standard. *Operculina turpethum* and Diclofenac sodium were administered 30 min prior to intra-peritoneal administration of 0.6% acetic acid. Percentage protection against writhing was taken as an index of analgesia. The results were tabulated in table.2 and expressed as Mean ± SEM of four animals in each group.[12] (The results were tabulated in table 2)

### It is calculated as:

$$\frac{\text{Number of writhing in control group} - \text{Number of writhing in treated group}}{\text{Number of writhing in control group}} \times 100$$

**Table 1: Phytochemical screening of different solvent extracts of *Operculina turpethum***

| S.No | Chemical test | Petroleum ether extract | Chloroform extract |
|---|---|---|---|
| 1. | **Test for alkaloids:** | | |
| | (a) Mayer's test | - | + |
| | (b) Hager's test | - | + |
| | (c) Wagner's test | - | + |
| | (d) Dragendroff''s test | - | + |
| 2. | **Tests for Glycosides:** | | |
| | (a) Anthraquinone glycosides | - | + |
| | (i) Borntrager's test | - | + |
| | (b) Cardiac glycosides | | |
| | (i) Keller-killiani test | - | + |
| | (ii) Legal's test | - | + |
| | (iii) Baljet test | - | + |
| 3. | **Tests for carbohydrates** | | |
| | (a) Molisch's test | - | + |
| | (b) Barfoed's test | - | + |
| 4. | **Tests for reducing sugars:** | | |
| | (a) Fehling's test | - | + |
| | (b) Benedict's test | - | + |
| 5. | **Test for Proteins:** | | |
| | (a) Xanthoprotein test | - | - |
| | (b) Precipitation test with | | |
| | (i) Lead acetate | - | - |
| | (ii) Copper sulphate | - | - |
| 6. | **Test for Steroids:** | | |
| | (a) Salkowski test | + | + |
| | (b) Sulphur powder test | + | + |
| 7. | **Tests for Flavonoids:** | | |
| | (a) Shinoda test | - | + |
| | (b) Test with Lead acetate | - | + |
| | (c) Alkaline reagent test | - | + |
| | (d) Dilute ammonia test | - | + |
| 8. | **Tests for tannins & phenolic compounds:** | | |
| | (a) Test with ferric chloride | - | - |
| | (b) Test with Lead acetate | - | - |
| | (c) Teat with Bromine water | - | - |
| | **Tests for volatile oils:** | | |
| 9. | (a) Odour | + | + |
| | (b) Filter paper stain test | + | + |
| | (C) Solubility test | + | + |

**Table 2: Analgesic activity of different solvent extracts of *Operculina turpethum* and Diclofenac sodium using mice by acetic acid induced writhing method**.

| S.No | Groups | Treatment | Dose | No. of writhing responses with in 20 min. | % inhibition |
|---|---|---|---|---|---|
| 1 | Group I | Normal saline | 0.2ml | 72.3 ±1.453 | - |
| 2 | Group II | Diclofenac sodium | 10mg/kg | 17.3 ± 1.453 | 76 |
| 3 | Group III | Chloroform extract | 125mg/kg | 53.6 ±0.882 | 25.8 |
| | | | 250mg/kg | 46.3 ±0.882 | 35.9 |
| | | | 500mg/kg | 30.3 ±0.882 | 58 |
| | | | 1000mg/kg | 21.3 ± 0.667 | 70.5 |
| 4 | Group IV | Pet ether extract | 125mg/kg | 62.3 ± 1.453 | 13.8 |
| | | | 250mg/kg | 50.3 ± 0.667 | 30 |
| | | | 500mg/kg | 34.3 ± 1.453 | 52.5 |
| | | | 1000mg/kg | 28.3 ± 1.453 | 60.8 |

**Mean ± SEM values (N = 4)**

**Tail immersion method**

Mice were divided into four groups, four animals in each. Group I served as vehicle control receiving normal saline. Group II served as positive control Diclofenac sodium (10 mg/kg, i.p) was given. Groups III & IV received chloroform and petroleum ether extracts of *Operculina turpethum* (125, 250, 500 and 1000 mg/kg).

The distal 2 - 3 cm portion of mouse-tail was immersed in hot water maintained at 55 ± 0.5ºC. [109] The time taken by the mouse to withdraw the tail from hot water was noted as reaction time. The reaction time was noted at 0, 30, 60, 90,120 and 150 min. The results were tabulated in table.3 and expressed as Mean ± SEM of four animals in each group. [13] (The results were tabulated in table 3)

Fig1: Writhing Method

Fig2: Tail Immersion Method

**Table 3: Analgesic activity of different solvent extracts of Operculina turpethum and Diclofenac sodium using mice by tail immersion method.**

| S.No | Groups | Treatment | Dose | Basal reaction time (s) (mean ± SEM) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0 min | 30 min | 60 min | 90 min | 120 min | 150 min |
| 1 | Group I | Normal saline | 0.2ml | 1.76 ± 0.033 | 1.63 ± 0.033 | 1.46 ± 0.033 | 2.2 ± 0.058 | 2.23 ± 0.088 | 1.63 ± 0.088 |
| 2 | Group II | Diclofenac sodium | 10mg/kg | 1.43 ± 0.067 | 1.5 ± 0.058 | 2.4 ± 0.058 | 4.1 ± 0.088 | 5.1 ± 0.058 | 3.5± 0.058 |
| 3 | Group III | Chloroform extract | 125mg/kg | 1.3 ± 0.058 | 1.63 ± 0.088 | 2.33 ± 0.145 | 2.43 ± 0.12 | 2.67 ± 0.145 | 3.26 ± 0.12 |
| | | | 250mg/kg | 1.5 ± 0.058 | 2.26 ± 0.12 | 2.46± 0.203 | 3.66 ± 0.088 | 3.63 ± 0.088 | 3.2 ± 0.058 |
| | | | 500mg/kg | 1.46± 0.088 | 1.73 ± 0.088 | 1.96 ± 0.12 | 2.3 ± 0.088 | 3.45 ± 0.088 | 2.7 ± 0.115 |
| | | | 1000mg/kg | 1.2 ± 0.0115 | 1.4± 0.058 | 1.5 ± 0.115 | 1.8 ± 0.153 | 2.56 ± 0.176 | 3.36 ± 0.145 |
| 4 | Group IV | Pet ether extract | 125mg/kg | 2.26 ± 0.12 | 2.56± 0.12 | 3.43 ± 0.12 | 3.5± 0.173 | 3.86 ± 0.176 | 2.3 ± 0.145 |
| | | | 250mg/kg | 1.33 ±0.088 | 1.33 ± 0.088 | 2.6 ± 0.115 | 3.26 ± 0.12 | 3.43 ± 0.12 | 3.4 ± 0.115 |
| | | | 500mg/kg | 2.26± 0.12 | 2.43 ± 0.12 | 3.2 ± 0.115 | 4.26± 0.12 | 4.63 ± 0.088 | 3.2 ± 0.088 |
| | | | 1000mg/kg | 2.13 ± 0.088 | 2.66 ± 0.2 | 2.68 ± 0.11 | 3.23 ± 0.145 | 3.83 ± 0.088 | 3.56 ± 0.145 |

**Mean ± SEM values (N = 4)**

**RESULTS**

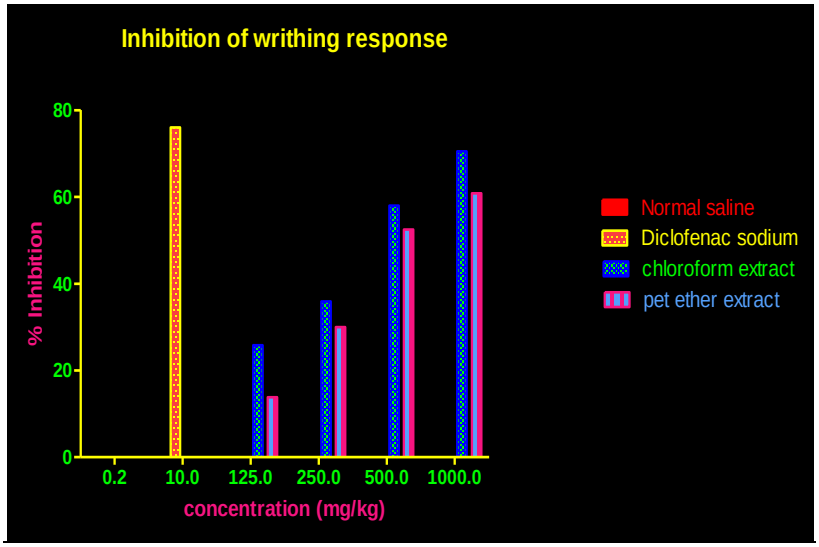


**Fig 9: Comparison of % inhibition of different solvent extracts of Operculum turpethum and Diclofenac sodium using mice by acetic acid induced writhing method**

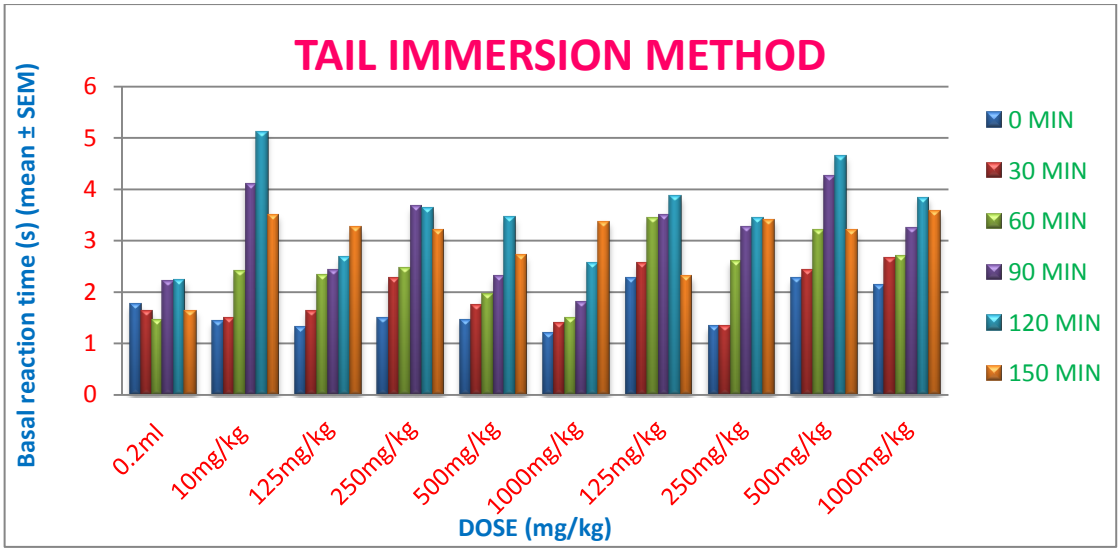


**Fig 10: Comparison of analgesic effect of different solvent extracts of Operculina turpethum and Diclofenac sodium using mice by tail immersion method.**

## DISCUSSION

From the observations made, The Phytochemical screening in this study revealed that the chloroform extract contains alkaloids, carbohydrates, reducing sugars, proteins, sterols, flavonoids, tannins, phenols and volatile oils and petroleum ether extract contains sterols and volatile oils. The presences of volatile oils are mainly responsible for their perfumery actions.

Acetic acid induced writhing in mice attributed visceral pain finds much attention of screening analgesic drugs. [14] Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipid [15] via cyclooxygenase (COX), and prostaglandin biosynthesis). In other words, the acetic acid induced writhing has been associated with increased level of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products.[16] The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability. The acetic acid induced writhing method was found effective to evaluate peripherally active analgesics. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition. The significant pain reduction of both the plant extracts might be due to the presence of analgesic principles acting with the prostaglandin pathways.

The analgesic activity of this study revealed that the different solvent extracts of the plant shows activity against various types of pain stimuli. In case of acetic acid induced writhing method the % inhibition (70.5% for 1000mg/kg) produced by chloroform extract is more when compared to petroleum ether extract (60.8% for 1000 mg/kg). The % inhibition of writhing response increases with increasing the concentration of the plant extracts. The standard drug exhibit highest % inhibition (76% for 10 mg/kg) when compared to plant extracts. The order of potency is Diclofenac > chloroform > petroleum ether > normal saline.

In case of tail immersion method, the heat itself acts as a source of pain. The different concentrations of chloroform and petroleum ether extracts of plant (125, 250, 500, 1000 mg/kg) and diclofenac sodium (10 mg/kg) was administered to mice and observe the basal reaction time in different time intervals. The basal reaction time increases with increasing the concentrations along with increasing the time. The basal reaction time is more for standard drug when compared to plant extracts. In case of plant extracts the petroleum ether extracts shows more reaction time and that is recovered after 150 min. the order of potency is diclofenac sodium > petroleum ether > chloroform > normal saline.

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