# PHARMACOLOGICAL SCREENING OF HYPERICUM OBLONGIFOLIUM FOR ITS ANALGESIC EFFECT IN MICE

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## ABSTRACT

**Objective:** The present study is aimed to determine the anti-nociceptive activity of crude methanol extract of Hypericum oblongifolium.

**Material and Methods:** In vivo acetic acid induced writhing test was used for anti-nociceptive effects in mice at 10, 20 and 30 mg/ kg body weight respectively intraperitoneally.

**Results:** The extract doses of 10, 20 and 30 mg/kg revealed significant inhibitory effect ($P < 0.001$) in acetic acid induced writhing test.

**Conclusions:** The methanol extract of medicinal plant Hypericum oblongifolium showed significant analgesic / anti-nociceptive effects in animal models and thus supports the traditional uses of the plant in painful conditions.

**Key Words:** Hypericum oblongifolium, Methanol extract, Anti-nociceptive activity.

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## INTRODUCTION

American Pharmacopoeia (1820) contained more than six hundred drugs, and approximately 70 %, (more than four hundred) were from plant origin, while the eleventh edition of the same Pharmacopoeia (1936), contained about 45% drugs of plant origin[1].The Pharmacological effects of these herbal products, as evident from phytochemical studies, are due to active compounds in these plants. It has been established by W.H.O. that approximately 80% of the population is using these herbs as traditional medicines [2]. The International Association for the Study of Pain (IASP) has defined Pain as a disagreeable and disturbing experience due to tissue damage[3]. Pain is one of the most important symptoms. A large number of commercial preparations, like NSAIDs, are available, playing a vital role in relieving pain and inflammation. Their useful effects are due to inhibiting cyclooxygenase enzymes[4]. Cyclooxygenases act on Arachidonic acid and cause synthesis of Prostaglandins. Aspirin, Ibuprofen, Diclofenac and Naproxen are common examples of NSAIDs. Analgesics relieve painful symptoms only without treating the underlying cause[5]. But most of these compounds have adverse effects like dyspepsia, gastrointestinal bleeding, and peptic ulcer[6]. In order to avoid these adverse effects, a large number of medicinal plants are investigated for their potential analgesic activity. Some of these plants include Ligusticum porter, used in headache and Brickellia veronicaefolia used in arthritic pain[7]. Species of Mexican traditional folk medicinal plant genus Gnaphalium are still used for its beneficial effects in relief of lumbago, fevers, and other inflammatory conditions, and Amphipterygium adstringens is used as anti-inflammatory, analgesic, anti-pyretic and antibiotic[8].

Hypericum oblongifolium, belongs to the family Hyperiaceae, is a shrub which grows at a height of approximately 5000-5500 ft, and is common in China and Indo-Pak regions. The flowers are yellow, having persistent-withering petals, bloom from June till September[10]. Phytochemical analysis of the flowers showed the presence of flavonoids, saponins and tannins in Hypericum oblongifolium[11]. Other active principles of the plant are Flavonols, Phenolic compounds and Essential oils[12]. One of the many analgesic ingredients of the medicinal plants is Flavonoids. These are compounds which cross the blood-brain barrier and affect the pain through different mechanisms in the central nervous system. These may act like opium alkaloids or GABA, or may act though their receptors. Another mechanism may be by inhibiting the enzymes involved in the process of inflammation[13]. Researches show that they may decrease the Calcium ion concentration in the cells, by inhibiting the activity of N-methyl-D-aspartate receptors. They may inhibit the

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activity of the enzyme, responsible for synthesis of nitric oxide (NO) and prostaglandins, which are the mediators causing inflammation and pain[14]. The Flavonols have got astringent, anti-viral and anti-inflammatory activity[15] on one hand and sedative, diuretic, anti-inflammatory and anti-diarrheal activity on another hand[16].

This study is intended to determine the anti-nociceptive activity of crude methanol extract of Hypericum oblongifolium. If found effective, the results will be shared with local physicians to use it especially in cases where the traditional NSAIDs are contraindicated.

## MATERIAL AND METHODS

### *ANIMALS*

Mice weighing 20-25 gm were supplied by National Institute of Health Islamabad and kept in the animal house of Pharmacy department, university of Peshawar. They were used for analgesic studies. They were kept in groups in their cages, each having eight animals. Cages were having solid bases and saw dust beddings. Standard animal food and water supplied. They were kept in 12 hr light/dark cycle. Laboratory temperature was maintained at 22 ± 2° C.

### *PLANT MATERIAL*

Hypericum oblongifolium, is a natural herb of the Hilly areas of Khyber Pakhtunkhwa, (NARAN, and GALLY-AT areas). With the help of a Taxonomist, plant was identified and collected.

### *CHEMICALS/DRUGS*

Aspirin (Acetyl salicylic acid) was obtained from Oval Pharmaceuticals, Lahore Pakistan, while disposable micro-syringes, Acetic acid, Saline (0.9% NaCl), Methanol, Chloroform, and Cotton were purchased from local market.

The aerial parts of the plant (flowers, leaves and stem) were cleaned and shed dried. After drying they were powdered in the electric rotator. A weighed amount (500 gm) of the dried powder was dissolved in 80% methyl alcohol at room temperature and was kept undisturbed for one week with daily shaking at different timings. The extract was filtered and was shifted to rotary evaporator and a semi-solid dark crude extract was obtained with 23% yield.

### *INTRAPERITONEAL ADMINISTRATION*

Normal saline, Acetic acid, Aspirin, and extract of Hypericum oblongifolium were administered through intraperitoneal route to mice. The ventral surface (abdomen) of the animal was exposed, by holding the skin behind the neck firmly, putting the tail behind the little finger of the left hand. The needle was inserted directly into the peritoneum of the animal. The drugs / extracts were injected in the cavity with right hand.

### *EXPERIMENTAL PROCEDURES*

### *ANALGESIC ACTIVITY*

### *WRITHING TEST*

Abdominal constrictions induced by acetic acid, the WRITHING TEST, in animals is the common model test for observation and assessment of peripheral analgesic effect[17, 18]. In this test, Acetic acid (1%) was used as a chemical stimulus to elicit painful sensation in the experimental animals. The animals (mice) were kept in different chambers and the abdominal constrictions were observed for 30 minutes post administration of acetic acid injected intra-peritoneal. A Writh or abdominal constriction is adoption of a peculiar position of animal with flat abdomen, depressed back and extended hind limbs. The adaptation of this posture, called writh or abdominal constriction is then followed by normal posture [19].

The extract of Hypericum oblongifolium was tested for analgesic activity in animal (Swiss mice) model of analgesia, i.e. Writhing test. Animals were exposed to a 12-hours light/dark cycles (lights on 0800). Experiments were performed during the light part. Before the start of the experimental procedure, food was withdrawn for 12 hours, but water was given. The animals were tested individually in two compartment box, so that two animals could be observed at a time.

### *DOSE PREPARATION*

Acetic acid 1% saline. 0.1 ml of glacial acetic acid (100% extra pure) was diluted in 10 ml normal saline.

Aspirin solution (3mg/ml). 60 mg of Aspirin dissolved in 20 ml of normal saline.

Normal Saline. 0.9 % solution of Sodium chloride.

Hypericum oblongifolium Extract solution (5mg/ml). 50 mg of the Methanolic extract was dissolved in normal saline to make 10ml solution.

### *PROCEDURE*

Acetic acid (1%) was given intraperitoneally to the animals. Animals were kept in observation chambers and the number of writhes in the 30 minutes period was counted. Animals were grouped into various treatment groups as follows;

Saline Control Group (n=6)

Each animal in this group received Saline (sodium chloride) 10 ml/kg body weight intraperitoneal.

Aspirin Group (n=18)

This group was having 3 sub-groups A, B, and C (n=6) and received Aspirin in 25mg /kg body wt, 50 mg / kg body wt, and 100mg /kg body wt respectively. Aspirin was given by intra peritoneal route 45 minutes prior to acetic acid administration.

Hypericum oblongifolium treatment Group (n=18)

This group was having 3 sub-groups each having 6 animals, and the dose schedule of Hypericum oblongifolium, was 10 mg/kg body wt, 20 mg/kg body wt, and 30 mg/kg body wt respectively. The extract was given through Intra-peritoneal route forty-five minutes prior to acetic acid administration. The percentage protection was obtained for the control as well as drug treatment groups with the help of the following formula.

% Protection = Mean Control group – Mean drug group divided by Mean Control group × 100

### *STATISTICAL ANALYSIS*

The data collected, was expressed as means ± S.E.M. ANOVA with Dunnet post hoc test, applied to the data to check the significance

## RESULTS

### *A. ANALGESIC ACTIVITY OF ASPIRIN*

Table 1, shows that different doses of Aspirin, injected to mice, has significantly decreased that number of contractions, WRITHES

Percent protection.

Saline = zero percent

Aspirin (25mg/kg body weight) = 50.23%

Aspirin (50mg/kg body weight) = 71.87%

Aspirin (100mg/kg body weight) = 85.1%

Fig. 1 shows the analgesic activity produced by giving Aspirin, at different doses of 25, 50, and 100 mg per kg body weight. The column shows the number of writhes (MEAN ± SEM) of mice. Aspirin has significantly (***p< 0.001) decreased the number of acetic acid induced contractions and produced analgesic effects as compared to control group.

### *B. ANALGESIA PRODUCED BY HYPERICUM OBLONGIFOLIUM EXTRACT*

Table 2 shows the percent protection of different doses of plant extract (Hypericum oblongifolium) on Acetic acid induced abdominal constriction/ writhes. Different doses of plant extract given to mice, has significantly decreased the number of contractions, WRITHES

Percent protection.

Saline = zero percent

Hypericum oblongifolium (10mg/kg body weight) = 8.65 %

Hypericum oblongifolium (20mg/kg body weight) = 52.64%

Hypericum oblongifolium (30mg/kg body weight) = 68.03%

Fig.2: Analgesia Produced By Hypericum Oblongifolium Extract

Fig. 2 shows the analgesic effect produced by administering plant extract, at doses of 10, 20, and 30 mg/kg body weight. The columns show the number of writhes, (MEAN ± SEM of mice).

The Plant extract has significantly (**p< 0.01) decreased the number of acetic acid induced writhes, and produced analgesic effects as compared to control group.

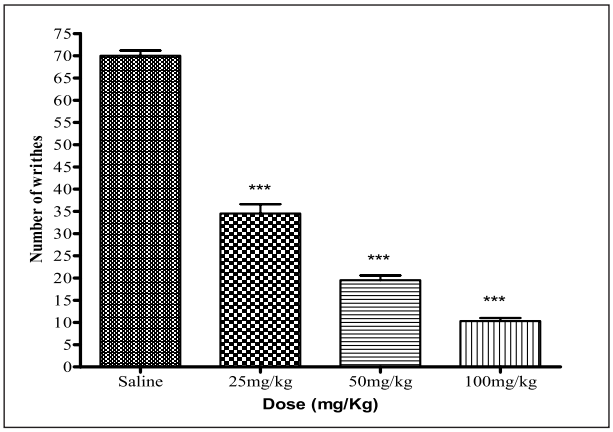


**Fig 1: Analgesic effect of Aspirin**

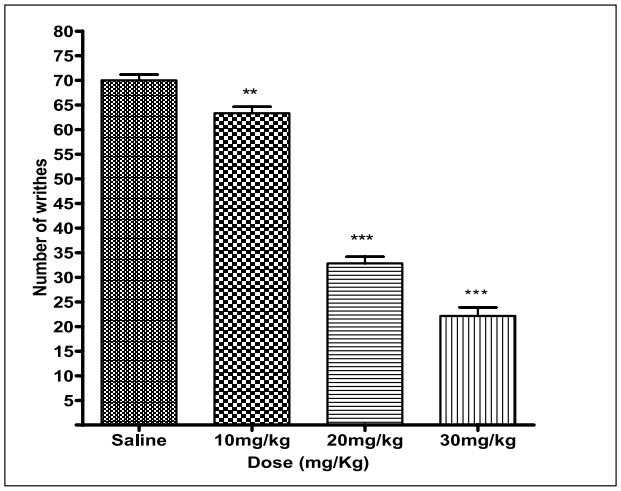


**Fig 2: Analgesic effect of Hypericum oblongifolium extract**

**Table 1: Analgesic activity of Aspirin**

| No | Acetic acid | Saline | Aspirin 25mg/kg | Aspirin 50mg/kg | Aspirin 100mg/kg |
|---|---|---|---|---|---|
| 1 | 66 | 66 | 36 | 18 | 12 |
| 2 | 67 | 68 | 42 | 23 | 10 |
| 3 | 70 | 71 | 38 | 18 | 8 |
| 4 | 72 | 74 | 28 | 20 | 11 |
| 5 | 68 | 69 | 30 | 16 | 9 |
| 6 | 73 | 72 | 33 | 22 | 12 |
| Mean | 69.33 | 70.0 | 34.5 | 19.5 | 10.33 |
| + SEM | 1.15 | 1.18 | 2.13 | 1.09 | 0.67 |

**Table 2: Analgesic activity of Hypericum oblongifolium extract**

| No | Acetic acid | Saline | H.oblongifolium 10mg/kg | H.oblongifolium 20mg/kg | H.oblongifolium 30mg/kg |
|---|---|---|---|---|---|
| 1 | 66 | 66 | 60 | 38 | 20 |
| 2 | 67 | 68 | 60 | 35 | 21 |
| 3 | 70 | 71 | 62 | 30 | 19 |
| 4 | 72 | 74 | 68 | 32 | 30 |
| 5 | 68 | 69 | 64 | 29 | 24 |
| 6 | 73 | 72 | 66 | 33 | 19 |
| Mean | 69.33 | 70.0 | 63.33 | 32.83 | 22.16 |
| + SEM | 1.15 | 1.18 | 1.22 | 1.35 | 1.74 |

## DISCUSSION

The Analgesic activity of medicinal plant Hypericum oblogifolium was evaluated experimentally by using the standard Writhing test in mice.

Non-Steroidal Anti Inflammatory Drugs produce their analgesic and anti-inflammatory effects by inhibition of the enzyme cyclo-oxygenase[4]. It is stated that injection of a chemical substance Acetic acid in the peritoneum of the animal has resulted in liberation of the mediators of inflammation. These mediators are named as 5-Hydroxytryptamine, (also known as Serotonin), Histamine, Prostaglandins, Bradykinins, and substance P. These mediators, via exciting pain receptors cause pain[20, 21, 22]. It is proved that the major inflammatory mediators are Prostaglandins, and Bradykinins, and they act through cyclooxygenase pathway [23, 24, 25].

The medicinal plants have active principles like Flavonoids and Tannins which are responsible for prevention of synthesis of the inflammatory mediator prostaglandin[26]. The medicinal plant, Hypericum oblongifolium contain flavonoids and tannins[12] and it is possible that these two active principles of the plant may be responsible to inhibit the formation of the inflammatory mediators i.e. Prostaglandins and Bradykinins. A study of another member of this family i.e. Hypericum perforatum, has shown analgesic activity[27]. It is also proved that Luteolin and myricetin, which are flavonoid aglycones by nature, are present in the plant Hypericum oblongifolium [28], and they have proved analgesic [29], anti-inflammatory [30], and antipyretic activities [31].

The analgesic and anti-inflammatory properties of the plant may be either due to presence of these flavonoids or other active principles. They are responsible for inhibition of synthesis of these mediators which cause pain and inflammation. The present study has revealed that the plant has produced analgesia, and it is possible that the flavonoids and other active principles present in the plant have similar mechanism of action as that of commonly used analgesics. The exact mechanism of analgesia and anti-inflammatory effect, need further studies, but this study shows that the analgesic and anti-inflammatory effects of the plant may either be due to the presence of these active principles or some other mechanisms are involved which needs detail studies. However, the present study provides a scientific basis for other researchers to assess and screen these medicinal plants for possessing analgesic and anti-inflammatory activities.

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**AUTHOR'S CONTRIBUTION**

Following authors have made substantial contributions to the manuscript as under

| | |
|---|---|
| **Ali J:** | Data collection, Analysis, and proof reading. |
| **Ali F:** | Data collection, manuscript writing, Methodology. |
| **Khan A:** | Data collection, Manuscript writing, data analysis conclusion. |
| **Junaid M:** | Manuscript waiting Methodology, data analysis table and graphs. |

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.