



EVALUATION OF *TRICHOLEPIS GLABERRIMA* FOR ANALGESIC ACTIVITY USING IN VIVO MODELS

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ABSTRACT

The main objective of present study was to evaluate the analgesic activity of the 95% w/v methanolic extract of *Tricholepis glaberrima* (METG) a plant used as traditional remedy and popularly known as "Bhramdandi" belongs to the family Asteraceae and was screened for various pharmacological activities. The analgesic effect was examined by two methods the Eddy's Hot plate method and Tail immersion method at the doses of 100, 200, 400 mg/kg. The results showed 200 and 400 mg/Kg of the extract were more significant and comparable with the effect of diclofenac sodium 30mg/kg. The results of this study demonstrated the analgesic effect of METG extract in animal models which showed that the latency period of METG extract was almost equal at 0 min and gradually increased from 60 min, peaking at 90 min and justified traditional use of this plant in the treatment of pain. More studies are needed to elucidate the active components.

KEYWORDS: *Tricholepis glaberrima* (METG).

INTRODUCTION

Pain may arise from diverse etiologies. It can be due to the direct effects of a disease or by its treatment even surgery, radiation, and therapy may all result in pain. The patient may also have chronic underlying disease that directly causes or contributes to pain. Diseases such as osteoarthritis, cancer, neuropathies, and vascular insufficiency can cause chronic pain. Pain can also be caused due to metabolic disease, infection, ischemia, injury, entrapment, connective tissue disease, acquired immunodeficiency, malignancy, drugs, and toxins.^[1,2] Since different modalities may be required to effectively treat the variable causes of pain, identifying and quantifying all of the different causes of pain that affect the patient will help to determine the appropriate therapy.^[3]

Recent studies have found that some people with chronic pain may have low levels of endorphins in their spinal fluid.^[4] Synthetic drugs that are employed for managing pain are readily available but are associated with adverse effects. Therefore the use of herbal medicine is an age old practice used to cure diseases.^[5] These medicinal plants contain phytochemical compounds capable of relieving pain. However, an extensive search of the literature reveals that there is a limited data documented about the therapeutic use of the herbal medicine in suppressing pain.

Tricholepis glaberrima popularly known as "Bhramdandi" belongs to the family Asteraceae. It was screened for various pharmacological activities like antioxidant^[6], aphrodisiac^[7] nerve tonic^[8], Anti depressant activity^[9], hepatoprotective^[10] and Anti inflammatory^[11] effects in experimental animal models. Aerial parts of *Tricholepis glaberrima* used in several types of diseases. Thus, the present study was designed to screen the antidepressant activity of aerial parts of *Tricholepis glaberrima*.

Experimental Animals and Housing of Animals

The protocol was approved by the institutional animal ethics committee of SSJ COLLEGE OF PHARMACY and the approval registration number is: 1448/PO/Re/S/11/CPCSEA/01/2016.

Albino Wistar rats weighing 150 ± 25 g of either sex were used for the study in different models. Animals kept in well-maintained room under standard hygienic conditions. Commercial pellet diet and water were made available. They were housed in propylene cages (32 x 24 x 16 cm) with stainless steel grill top, bedded with rice husk.

METHODOLOGY

The leaves of *Tricholepis glaberrima* were dried under shade in room temperature for 3 days and powdered and

the powder was used for preparation of methanolic extract. A 95% w/v methanolic extract was prepared by Soxhlet extraction method. The extract was concentrated at 400 °C to obtain light brown residue. The yield obtained from above process was found to be 9.5%. The extract was preserved in a refrigerator for further use.

Based on acute toxicity studies the lethal dose was found at 2000mg/kg body weight, the 1/10th of the preceding dose i.e. 100 mg/kg body weight was taken as the testing dose for this study and consequently 200 and 400 mg/kg body weight also tested to find out dose dependent pharmacological effect.

Screening of analgesic activity

Two methods i.e., eddy's hot plate method and tail immersion method were employed for screening analgesic activity

Albino Wistar rats weighing between 150 -200 gms were divided into 5 groups of 6 rats each; three animals being housed in labeled cage each. Animals were given a period of time to adjust to the new environment provided with food & water ad libitum

Grouping

Group-I animals received 0.1 ml normal saline i.p which served as control. Group-II diclofenac sodium 30mg/kg while Groups-III, IV and V animals received methanolic extract of *Tricholepis glaberrima* (METG) at a dose of 100, 200 and 400 mg/kg respectively. Following administration of the samples, reaction time was measured at 0, 60, 90, 120 and 180 min.

Procedure

In eddy's hot plate method a transparent glass cylinder is used to keep the animal on the heated surface of the plate. The temperature of the hot plate is set using a thermo regulated water circulated pump. The time of latency is defined as the time period between the zero point, when the animal is placed on the hot plate surface, and the time when the animal licks its paw or jumps off to avoid thermal pain. The time for licking paws or jumping in hot plate was recorded as response, prior and 0, 30, 60, 90 120 min after administration of respective drugs.

In tail immersion method the animals were screened for sensitivity by immersing the tail of the rats gently in hot

water maintained at 55 - 55.5 °C. The rats that lifted their tails from the hot water within 5 s were selected for the study. The selected rats were then divided into four groups (n = 6). All the data's were analyzed using One-Way ANOVA method followed by Dunnet's test. All values were reported as mean ± SEM. P≤0.05 was considered to be statistically significant.

RESULTS

The methanolic extract was subjected to preliminary phytochemical analysis and the observations are presented in Table No. 1.

Table No. 1: preliminary phytochemical analysis of *Tricholepis glaberrima*.

Phytoconstituents	Present or Absent
Carbohydrates	Absent
Glycosides	Present
Fats	Absent
Gums & mucilages	Absent
Proteins & amino acids	Present
Saponins	Present
Tannins & Phenolic compounds	Present
Phytosterols	Present
Flavonoids	Present
Alkaloids	Present

5.3. Analgesic activity

Eddy's hot plate

Tricholepis glaberrima showed maximum analgesic activity at 60, 90 min for 100 and 200mg/kg dose. The reaction time in normal control group at 60, 90 min was found to be 0.77±0.22, 0.78±0.23 sec. The reaction time (paw licking / jumping response) in rats pretreated with lower dose of *Tricholepis glaberrima* (100mg/kg), higher dose of *Tricholepis glaberrima* (400mg/kg/day) and Diclofenac sodium (9 mg/kg) at 60, 90 min were found to be 2.82±0.52, 2.98±0.36, 4.02±1.02 and 4.58 ± 0.36 respectively when compared to control group rats.

The duration of analgesic effect was more in 200 mg/kg compared to 100 mg/kg and reference drug diclofenac sodium at 9 mg/kg dose significantly increased the reaction time at 90 minutes as shown in Table.1.

Table No. 2: Effect of *Tricholepis glaberrima* on reaction time (sec) in Eddy's Hot Plate

Groups	Dose	Reaction time in seconds				
		0	60	90	120	180
Control		0.78 ± 0.23*	0.77 ± 0.22*	0.78 ± 0.2*1	0.8 ± 0.18*	0.78 ± 0.22*
Std	50 mg/kg	0.82 ± 0.22	2.82 ± 0.52	4.02 ± 1.02	5.23 ± 1.04	7.55 ± 1.66
METG-1	100mg/k g	0.85 ± 0.11*	1.55 ± 0.36**	2.36 ± 0.36**	3.58 ± 1.36**	4.88 ± 0.88**
METG-2	200mg/k g	0.88 ± 0.36*	2.58 ± 0.25**	3.25 ± 0.84*	5.68 ± 1.50**	6.00 ± 1.24**
METG-3	400mg/k g	0.75 ± 0.89*	2.98 ± 0.36***	4.58 ± 0.36**	6.89 ± 1.74**	9.5±1.84**

Values are expressed as (Mean±S.E.M) n=6; One way ANOVA followed by Dunnet's test. ** P< 0.001 Vs control, * P< 0.05 Vs control.

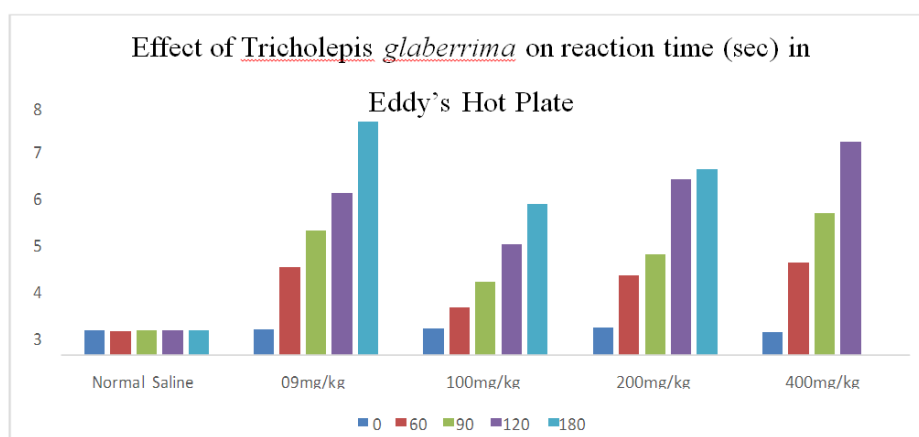


Figure 1: Effect of *Tricholepis glaberrima* on reaction time (sec) in Eddy's Hot Plate.

Table No. 3: Effect of *Tricholepis glaberrima* on reaction time (sec) in Tail Immersion Method.

Groups	Dose	Reaction time in seconds				
		0	60	90	120	180
Control		0.78 ± 0.23*	0.77 ± 0.22*	0.78 ± 0.21*	0.8 ± 0.18*	0.78 ± 0.22*
Std	50 mg/kg	0.82 ± 0.22	1.52 ± 0.12	2.02 ± 0.02	3.21 ± 0.84	4.05 ± 1.06
METG-1	100mg/kg	0.88 ± 0.14*	1.54 ± 0.36*	2.36 ± 0.36*	2.98 ± 1.05	3.78 ± 0.58*
METG-2	200mg/kg	0.89 ± 0.36*	2.58 ± 0.25*	3.25 ± 0.84**	3.98 ± 1.50**	4.00 ± 0.23**
METG-3	400mg/kg	0.78 ± 0.85*	3.08 ± 0.06**	4.05 ± 0.36**	4.40 ± 0.64**	4.92 ± 1.60**

Values are expressed as (Mean±S.E.M) n=6; One way ANOVA followed by Dunnet's test. ** P< 0.001 Vs control, * P< 0.05 Vs control

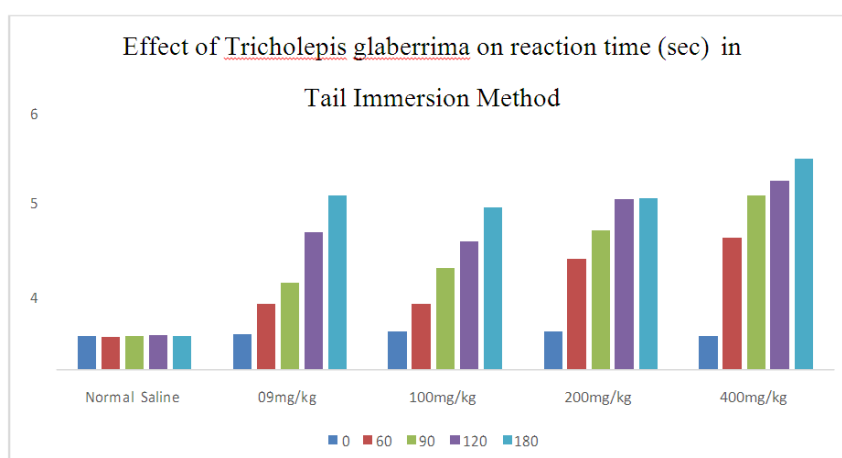


Figure 3: Effect of *Tricholepis glaberrima* on reaction time(sec) in Tail Immersion Method.

DISCUSSION

The hot plate technique and tail immersion technique have been found to be appropriate for assessment of centrally acting analgesics.^[12] In the process of pain the initial phase of the oedema has been attributed to the release of histamine and serotonin, and is maintained during the plateau phase because of kinin like substances and the second accelerating phase of swelling due to the release of prostaglandin like substances.^[13] The present study may therefore be attributed to the ability of the *Tricholepis glaberrima* to inhibit various chemical mediators of inflammation like histamine and 5-HT during the initial phase.

In the present study *Tricholepis glaberrima* significantly increased the reaction time in hot-plate test suggesting its central analgesic activity; the probable mechanism could be by inhibition of prostaglandin synthesis. Prostaglandins play significant role in different phases of inflammatory reactions and elicit pain by direct stimulation of sensory nerve endings and also sensitize sensory nerve endings to other pain provoking stimuli.^[14] Statistical data obtained by the study revealed a significant improve in analgesic activity and the effect of 100,200 mg/kg and 400 mg/kg extracts of *Tricholepis glaberrima* were comparable to that of standard drug. The effectiveness of analgesic agents in the tail

immersion pain model is highly correlated with relief of human pain.^[15]

In both the models, when compared to control the latency period of METG extract was almost equal at 0 min and gradually increasing from 60 min, peaking at 90 min. And the percent analgesic activity of METG extract was gradually increasing from 60 min.

CONCLUSION

The findings in this study suggest that the *Tricholepis glaberrima* possess analgesic activity. The results have been obtained in carefully controlled experiments with laboratory animals where psychological factors can presumably be ruled out. In all the tests the responses have been assessed by actual measurement and not by subjective comparisons which may be influenced by the observer. Therefore the statistical validity of the findings has been proved and they provide a scientific foundation for the use of the biologically active ingredients of *Tricholepis glaberrima* in pain conditions and explain the clinical effectiveness of the *Tricholepis glaberrima*.

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