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Analgesic Properties of Fresh Leaf Juice of *Tridax Procumbens* Linn.

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ABSTRACT

Ayurveda is one of the traditional systems of medicine practiced in India and Sri Lanka. Medicines from Ayurveda are largely based upon herbal and herbomineral preparations. Pain is an unpleasant sensation that occurs whenever any tissue is being damaged. Most human diseases have a pain and inflammation components which lead to individuals seek medical attention. The present study was designed to investigate the Analgesic activity of oral administration of fresh leaf juice of *Tridax procumbens L.* in Swiss albino mice (*Mus musculus*). The analgesic activity was determined using acetic acid-induced writhing response. These experimental results suggest that fresh leaf juice of *Tridax procumbens* produced analgesic effect in mice.

Keywords: Analgesic, oedema, *Tridax procumbens,* mice & Ayurveda.

INTRODUCTION

Ayurveda is one of the traditional systems of medicine practiced in India and Sri Lanka and can be traced back to 6000 BC (Charak, Samhita, 1949). Medicines from Ayurveda are largely based upon herbal and herbomineral preparations and have specific diagnostic and therapeutic principles (Patwardhan and Hopper, 1992). The therapeutic efficacies of many indigenous plants, for various diseases have been described by traditional herbal medicine practitioners. Natural products are the source of synthetic and traditional herbal medicines which are still the primary health care system in some parts of the world. In the present investigation we have studied *Tridax procumbens* for its analgesic activity.

Analgesic drugs which are currently in use are either narcotics or non-narcotics which have proven toxic effects and relive a pain without affecting its causes (Mate *et al*., 2008*)*. These are associated with adverse side effects on skin, gastrointestinal, renal, hepatic, central nervous system (Simon, 1995; Suleyman *et al*., 2007). On the contrary, many medicinal plants had been used successfully since long time without any side effects (Ikram, 1983).

In this respect new drugs with improved pain management capacity and fewer side effects are being sought with urgency. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effect (Farnsworth, 1989; Eisner, 1990). The research of plants with alleged folkloric use as pain relievers, therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs (Elisabetsky *et al*., 1995).

Tridax procumbens Linn. Commonly known as 'Ekdandi' and Maxican Daisly is a native of topical America. It also grows wildly in tropical Africa, Asia and Australia and is available in all seasons and in most parts of country (The wealth of India, 1985). It is a perennial herb belonging to the family Asteraceae. Its leaves are used in the treatment of bronchial catarrh, dysentery and diarrhea and for preventing hair loss. The juice of its leaves possesses antiseptic, insecticidal and parasiticidal properties. It possesses a large numbers of chemical constituents which have been identified and isolated in flowers as well as other aerial parts of plants. (Saxena and Albert, 2005, Chantraine *et al*., 1998).

The people from rural area and the tribal's use fresh leaf of plant as a remedy against several ailments. The leaf juice and the whole plant juice of *Tridax procumbens* is used to treat cuts and wounds (Malhotra and Moorthy, 1973; Sharma *et al*., 1979; Pandey *et al*., 1981; Sharma and Malhotra, 1984; Sudhakar and Roll, 1985; Goel and Murgal, 1988), in diarrehoea and dysentery (Sebastian and Bhandari, 1984). It is also used in scorpion bite, stomachache (Jain, 1991) and toothache (Sudhakar and Rolla, 1985). From these uses it seems that it possesses either pain killing properties or antimicrobial properties or both. The bacteriostatic activity of *Tridax procumbens* was demonstrated (Deshmukh *et al*., 2007). In the present investigations the analgesic activity of *Tridax procumbens was studied in swiss albino mice.*

Pain

Pain is an unpleasant sensation that occurs whenever any tissue is being damaged, and it causes the individual to react to remove the pain stimulus (Guyton and Hall, 2006) to prevent exacerbation of further damage. Therefore it is a protective mechanism for the body. Pain can accompany many disease processes. It is a sign for the brain that something unwanted happening to the organs or tissue. It could be either a skin injury but it will involve some level of pain (Matosiuk *et al.,* 2007). Currently two main mechanisms of analgesic action are considered as the most useful in pain management.

Peripheral analgesic action

The first involves chain of the prostaglandins transformation with the enzyme Cox-2, specifically involved in the inflammation process, the cyclooxygenase of the second type (Cox-2) (Feldman and McMahan, 2000). Prostaglandin**s** and bradykinin play an important role in the pain process (Hirose *et al*., 1984). Cyclooxygenase-2 (Cox-2) is an enzyme which is involved in synthesis of prostaglandins from arachidonic acid (Phadke, 1988). Prostaglandins elicit pain by direct stimulation of sensory nerve endings and also sensitize sensory nerve ending to other pain provoking stimuli (Campbell, 1991). Substances inhibiting Cyclooxygenases (Most are acting nonspecifically on Cox-1 and Cox-2) are called Nonsteroidal Anti-inflammatory Drug (NSAID) (Uionne and Gordo, 1994).

Central analgesic action

The second mechanism involves blocking of transduction of the pain stimuli to the brain and backward to cortical cord with the use of the opioid receptors system (Besson, 1999; Curtis *et al*., 2000). There are three categories of pain receptors or nociceptors.

- Mechanical nociceptors – respond to mechanical damage such as cutting, crushing or pinching.
- Thermal nociceptors – respond to temperature extremes, especially heat.
- Polymodal nociceptors – respond equally to all kinds of damaging stimuli, including irritating chemicals released from injured tissue.

None of the nociceptors have specialized receptor structures, they are all naked nerve endings. All nociceptors can be sensitized by the presence of prostaglandins, which greatly enhance the receptor response to noxious stimuli. The centrally acting analgents are narcotics which inhibits these receptors.
Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems (Bensreti and Sewell, 1983; Terman and Bonica, 2000). Most human diseases have a pain and inflammation components which lead to individuals seek medical attention

(Merskey, 1986). As a consequence analgesic and anti-inflammatory drugs are among the most prescribed drugs in clinical practice (Lim and Yap, 1999).

The flavonoids such as luteolins and quercetine are present in flowers (Ali *et al*., 2001). The aerial parts (except flowering tops) possess various saturated and unsaturated fatty acids (Gadre and Gabhe, 1988). The plant also possesses phytosterols such as beta – sitosterol, campesterols and stigmasterol, which impart an anti-inflammatory property to it (Gadre and Gabhe, 1993).

Acetic acid induced writhing

Acetic acid induced writhing model of visceral pain (Vyklicky, 1979) is a very sensitive test for analgesic drug development. Writhes can be described as a wave of constriction and elongation passing caudally along the abdominal wall with twisting of the trunk and extension of the hind limbs in mice. This is due to nociceptive property of acetic acid (Surender and Majumdar, 1995).

Acetic acid induced pain sensation by triggering localized inflammatory response. Such pain stimulus leads to release of free arachidonic acid from tissue phospholipids (Ahmed *et al*., 2006). The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells (Ronaldo *et al.,* 2000), acid sensing ion channels (Voilley, 2004) and the prostaglandin pathways (Koster *et al*., 1959; Hossain *et al.,* 2006). The results of acetic acid induced writhing test showed highly significant decrease in writhing due to treatment of fresh leaf juice of *Tridax procumbens*. The results were parallel to Voveran treatment. These results indicate that *Tridax procumbens* is a strong analgesic drug and is acting peripherally probably interfering with prostaglandin pathways.

Phytochemical analysis of *Tridax procumbens* showed presence of flavonoids, alkaloids, tannins, steroids (Nia *et al*., 2003; Agrawal *et al*., 2009; Salahdeen *et al*., 2004; and Kale *et al*., 2008). The observed analgesic activity may be attributed to these compounds (Hasan *et al*., 2009). The role of tannins in antinociception was reported by Vanu *et al.,* (2006); Salawu *et al*., (2008). Flavonoids have been found to play an important role in the peripheral analgesic property through inhibition of Cox-2 the enzyme involved in prostaglandin synthesis (Hossinzadeh *et al*., 2002, Salawu *et al*., 2008, Hasan *et al*., 2009, Zakaria *et al*., 2007, Gupta *et al*., 2008). Flavonoids have antioxidant property. They inhibit NO (Olszanecki *et al*., 2002) and cause vasodilation which is one of the important mechanisms of antinociception (Naseri *et al*., 2005). These evidences suggest that *Tridax procumbens* is an effective analgesic drug inhibiting pain sensation through peripheral analgesic action

MATERIAL AND METHOD

Plant material

Fresh leaves of *Tridax procumbens* were collected from the garden. The plant was authenticated at the Department of Botany, Govt. Vidarbha Institute of Science and humanities, Amravati by Dr. P.Y. Bhogonkar. The leaves were washed with sterile water and blotted with sterile muslin cloth and leaf juice was squeezed from all leaf parts by hand crushing. The fresh leaf juice of *Tridax procumbens* was used for oral administration.

Animals

The albino mice (*Mus musculus*) were used. The animal were housed, in cages made up of plastic or Galvanized iron, with medium size approximately 290 × 220 × 140 mm and containing bed at the bottom. The husk was changed every day for cleanliness and good health of animals. The food and drinking water supplied *ad libitum* to the animals. The temperature of the rearing room was maintained in the range of 20 to 25^{0}C. The 12 hours of light and 12 hours of dark was maintained in the animal house. The animals were fed on commercially available pelleted (Amrut Mice Feed, Sangli) diet.

Healthy male and female albino mice (*Mus musculus*) of age three months weighing between 25 to 30 gm were used for the study. Experimentation was carried out with due permission of Institutional animal ethics committee of registered animal house of institute registration No. 1060/ac/07/CPCSEA.

Analgesic Study

Acetic acid induced writhing test:-

This test was carried out to evaluate the analgesic activity of fresh leaf juice of *Tridax procumbens* described as per the modified method of Koster *et al*., 1959 and Santos *et al*., 1995. For this purpose 0.25ml of 0.75% acetic acid solution (v/v 0.01 ml/10gm) was

injected intraperitoneally in each group. This camions contraction of abdominal muscles, accompanied by stretching of the hind limb, called as writhing and stretching.

I) Control group: - received 20 µl of 0.9% saline orally.

II) Experimental group: - received 50µl of fresh leaf juice of *Tridax procumbens,* orally.

III) Positive control group: - received 20µl (1.25mg/ml) of Voveran dispersible which was dissolved in distilled water, orally.

After 5 minute of administration of acetic acid, the number of writhing and stretching within next 5 min. was recorded. A reduction in the writhing number as compared to the control group was considered as evidence for the presence of analgesic activity, which was expressed as percent inhibition of writhing. The writhing and stretching made after a time intervals of 30 minute were counted for 5 minute each up to 2 hours.

Data were calculated according to the following formula.

$$\% \text{ inhibition} = \frac{Wc - Wt}{Wc} \times 100$$

Wc – Mean no. of writhings (Control)
Wt – Mean no. of writhings (Treated)

RESULTS AND DISCUSSION

Acetic acid induced writhing test:

Experimental group were exhibits the acetic acid-induced writhing responses in mice, indicative of the analgesic activity of fresh leaf juice of *Tridax* procumbens significantly($p<0.001$) reduced writhing responses induced by acetic acid when compared with the control group and with Voveran, fresh leaf juice of *Tridax* procumbens showed moderately significant ($p<0.01$).

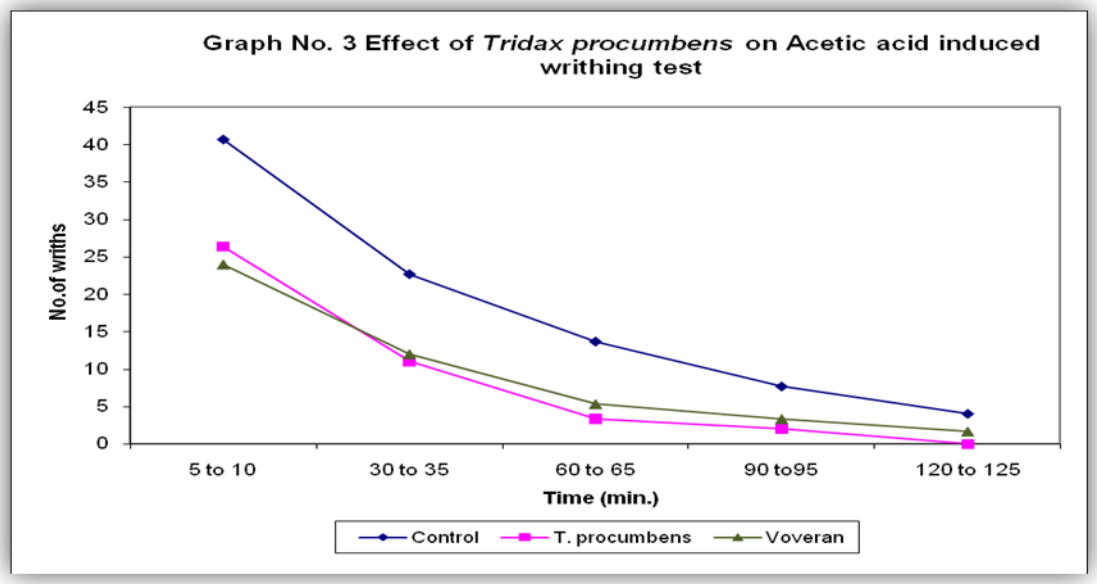
Table -: Effect of fresh leaf juice of *Tridax procumbens* on Acetic acid induced Writhing test.

Time (min.)	Control group	Experimental group	Positive control group
5 to 10	40.66 ± 7.23	26.33 ± 3.21*	24.00 ± 2.65**
30 to 35	22.67 ± 6.11	11.00 ± 1.00*	12.00 ± 3.00*
60 to65	13.66 ± 4.73	3.33 ± 1.15**	5.33 ± 2.08*
90 to 95	7.66 ± 0.58	2.00 ± 1.00***	3.33 ± 2.52*
120 to 125	4.00 ± 1.00	0	1.66 ± 0.58*,$$

(Average No. of writhes ± SD)

*=Statistically significant ($p< 0.05$) **,$$= moderately significant ($p<0.01$) ***=Highly significant ($p<0.001$)

*: Significance compared with Control $: Significance compared with



Two main mechanisms of the analgesic/ antinociceptive drugs are useful in pain treatment. The analgesic drug acting as inhibitors of Cyclooxygenase-2 are the peripherally acting analgents. For the study of analgesic effect of any drug acetic acid induced writhing test (koster *et al*., 1959).

Acetic acid induced writhing

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Conflict of Interest

The author declares that there is no conflict of interest.

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