

EXTRACT OF MORINGA OLEIFERA PLANT IN ALBINO WISTAR RATS".

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ABSTRACT

Introduction: Moringa Oleifera is widely found in Asian subcontinent and it has been used as an analgesic and anti-inflammatory in Indian folk medicine. In this study we compared the analgesic effects of Moringa Oleifera Ethanolic extracts with standard drug diclofenac in Wistar Albino Rats by hot plate method. **Methods:** Male Wistar albino rats were divided into 5 groups and administered placebo (saline), diclofenac and 3 groups of Moringa Oleifera using 100mg/Kg, 200mg/Kg and 400mg/kg doses. **Results:** Moringa Oleifera 400mg/Kg produced significant anti-nociceptive action by increasing prolongation of reaction time at 30 min, 60 min and 120 min. Moringa Oleifera (200mg/Kg) also produced significant antinocipetive action by prolongation of reaction time at 30 min, 60 min and 120 min. The Standard drug Diclofenac increased the prolongation of reaction time sustained all throughout the period **Conclusion:** Ethanolic extracts of Moringa Oleifera leaves exhibits significant anti-nociceptive activity by hot plate model in a dose dependent manner. However the amount of anti-nociceptive action produced was lesser as compared to standard drugs like Diclofenac.

KEYWORDS: Anti-nociception, Diclofenac, Moringa Oleifera.

INTRODUCTION

Moringa Oleifera belongs to Moringaceae family. There are 13 species of the family out of which *Moringa Oleifera* has become the most used and studied.^[1] Ayurvedic traditional medicine mentions that *Moringa Oleifera* can prevent 300 diseases and its leaves have been used both for preventive and curative purposes.^[2] Moringa is among the species utilized by traditional Siddha healers.^[3] Ancient Egyptians used *Moringa Oleifera* oil for its cosmetic value and skin preparation^[4]; even the species became popular among Greeks and Romans,^[5] *Moringa Oleifera* has been dubbed “miracle tree”, or “natural gift”, or “mother’s best friend”. In India, herbal drugs are an integral part of The Indian System of Medicine (Ayurveda) which is an ancient and mainstream system.^[6]

Moringa is one such species which has not been explored fully despite the enormous reports having potentials such as: cardiac and circulatory stimulants, antitumor, antipyretic, antiepileptic,^[7] anti-inflammatory, diuretic, antispasmodic,^[8] antiulcer^[9], antihypertensive, cholesterol lowering,^[10] antioxidant, antidiabetic, antitumor, hepato-protective, antibacterial and antifungal activities.^[11] These are also being used for treatment of different ailments in the indigenous system of medicine.^[12] The leaves are used in folklore medicine for the treatment of pain. Some previous reports indicate that

ethanolic extract of the leaves possesses significant anti-nociceptive activities. Therefore the present study is aimed to evaluate the analgesic activity of Ethanolic leaf extract of Moringa Oleifera plant in Albino Wistar Rats using Hot plate method.

MATERIALS AND METHODS

Plant Material and Extraction

Fresh leaves of *Moringa Oleifera* of were collected from periphery of Aurangabad city and its identity was confirmed by Department of Botany, Maulana Azad College Aurangabad. Leaves were dried in the shade inside the room for two days and later made into powder. 90% ethanol was used to extract the powder using the method of soxhlation for 18 hrs. Whitman filter paper No. 1 was used to filter the extract and concentrated to yield a semi solid mass of 48 gm (yield 9.2% w/w) and was refrigerated at 4°C and for later use .

Chemicals: Diclofenac (VOVERAN, NOVARTIS, Worli, Mumbai) and other solvent chemicals used were of analytical grade.

Animals

Animals were procured from central animal house of M.G.M. MEDICAL COLLEGE and Hospital Aurangabad. Male Wistar Albino rats (100-200 g) were used. Food, water given *adlibitum*. Animals were

acclimatized for laboratory conditions for 7 days before the experiments. The experimental study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of MGM Medical College and Hospital, Aurangabad constituted as per the guidelines laid by the committee for the purpose of control and supervision of experiments on Animals (CPCSEA).

Methods

30 Male Albino Wistar Rats were taken. They were divided into 5 groups of 6 animals each. First group of rats were considered as controls and treated with 0.2 ml normal saline orally. Second group were considered as standard and were treated with diclofenac orally, at dose of 10 mg/kg body weight, Third to fifth group were considered as test and treated with *Moringa Oleifera* at dose of 100 mg/Kg, 200 mg/Kg, 400mg/Kg, orally to find best analgesic action. Model of pain was induced by eddy's hot plate.^[13] Following administration according to respective grouping rats were placed on a hot plate maintained at $55 \pm 1^\circ\text{C}$. Latency of nociceptive response such as licking, flicking of a hind limb or jumping was measured. Measurements were performed at time 0 before and 30, 60 and 120 min after drug administration, with a cut-off time of 15 seconds to avoid lesions to the animals' paws.

RESULTS

In Group 1 there was no analgesic effect seen at 0, 30, 60 and 120 minutes. In group 2 the response to heat application was not seen at 0 minute interval but the prolongation of reaction time was slightly increased after 30 minutes which gradually increased after 60 and 120 minutes. In Group 3 at 0 minute there was no analgesic effect seen, but prolongation of reaction time increased at 60 and 120 min. In Group 4 the prolongation of reaction time effect was increased between 60 and 120 minutes. In Group 5 the analgesic prolongation of reaction time was not seen at 0 minutes but increased progressively from 30 minute to 60 min and up to 120 min.

The highest prolongation of reaction time was observed in Group 2 and Group 5 at 120 min. At all time of point, the prolongation of reaction time differed significantly between the extract and Diclofenac treated Standard groups being greater in the Group 2. Comparing different doses of the extract revealed that there is positive relationship between reaction time and increase dose of the extract in which, protection against thermal stimuli with 400 mg /kg was significant compared to all doses of the extract.

Table: Analgesic activity by hot plate method

Groups	0min	30min	60min	120min
Group 1[control]	1.61 \pm 0.12 ^{ns}	2.11 \pm 0.14 ^{ns}	2.16 \pm 0.12 ^{ns}	2.26 \pm 0.10 ^{ns}
Group 2[std]	1.56 \pm 0.12 ^{ns}	3.3 \pm 0.26 *	4.23 \pm 0.15**	8.98 \pm 0.26**
Group 3[mo100]	1.7 \pm 0.08 ^{ns}	2.58 \pm 0.20 ^{ns}	2.93 \pm 0.018*	3.23 \pm 0.16*
Group 4[mo200]	1.71 \pm 0.11 ^{ns}	2.8 \pm 0.18*	4.06 \pm 0.16**	7.16 \pm 0.17**
Group 5[mo400]	1.8 \pm 0.14 ^{ns}	4.1 \pm 0.43**	6.3 \pm 0.26**	7.88 \pm 0.45**

* $p < 0.01$ – significant, ** $P < 0.001$ - Highly significant, ns – not significant

DISCUSSION AND CONCLUSION

Considering the costs imposed to the society by the pain relief treatments, and having the knowledge about the numerous side effects of the available analgesics in the clinical practice, the need for new analgesic drugs with higher efficacy and fewer side effects seems imperative. The effect of the extract in our study by hot plate method response provides a confirmation of the analgesic effect of *Moringa Oleifera* leaf. In this method, duration of time for peak activity was longer for the extract (60 min) than for the standard drug (30 min), this time gap may be due to the time lag between drug entering the central compartment and distribution into the target site or formation of an active metabolites that are endowed with analgesic activity. This might also be the reason for the longer lasting analgesic activity of the extract at doses of 200mg/kg and 400mg/kg throughout the study period as compared to the standard. Better activity of 400 mg/kg of the extract compared to standard drug diclofenac in group 2, suggests that there may be other constituents that contribute for the analgesic activity of the extract.

In the hot plate method, a plate heated at a constant temperature produces two behavioral components that

can be measured in terms of their reaction times, namely paw licking and jumping. Both are considered to be supra-spinal integrated responses. The ability of the extract to increase latency confirms the analgesic activities of the extract. This test confirms the anti-nociceptive action of Ethanolic extract of *Moringa*, which could be due to inhibition of prostaglandin synthesis. There is also a possibility that the anti-nociceptive action of Ethanolic extract could be due to inhibition of cytokines like TNF- α , IL-1 β , IL-8.

The data of Ethanolic extract of *Moringa Oleifera* possesses analgesic properties, which are probably mediated by both central and peripheral inhibitory mechanisms as well as via inhibition of prostaglandin synthesis. Phytochemical ingredients like flavonoids, saponins, tannins, terpenoids present in the leaf could be contributed to its analgesic action.^[14] The plant can therefore offer a potential benefit in the management of pain disorders. However, further research is needed to explore the active ingredients contributing to its activities and elucidate the exact mechanism of action of this plant.

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