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# DEVELOPMENT AND EVALUATION OF ANALGESIC POLYHERBAL FORMULATION CONTAINING SOME INDIGENOUS MEDICINAL PLANTS

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## ABSTRACT

The present study explores the Polyherbal formulation for Analgesic activity of ethanolic leaves extracts of *Momardica charantia* Linn and *Azadirachta indica* A. Juss agaist experimental model of Eddy's hot plate method And Heat conduction method response in rats. The effective dose of the extract for analgesic activity was calculated from dose-response curve by using the Eddy's hot plate method And Heat conduction method response in rats. Polyherbal suspensions were prepared by the trituration method using a suspending agent and ethanolic dried extract of leaves. The formulation of *Momardica charantia* Linn and *Azadirachta indica* A. Juss were evaluated for analgesic activity. In both of Eddy's hot plate method And Heat conduction method response in rats writhing response method; the intraperitoneal administration of formulation F1 and F2 induced a significant analgesic activity in a dose-dependent manner respectively in the rats. Formulation F1 has shown significant analgesic effect than the F2 with compare of Diclofenac sodium as a standard drug. The plant may have the phytoconstituents which inhibit cyclooxygenase enzyme or act on central opioid receptors.

## KEYWARD

*Momardica charantia* Linn and *Azadirachta indica* A. Juss. F1, F2, Diclofenac sodium

## INTRODUCTION

Pain is an ill-defined, unpleasant, sensation usually evoked by an external or internal noxious stimulus. It is a warning signal and primarily protective in nature, but causes discomfort. Analgesics are the drugs that selectively relieve pain by acting on the CNS (central nervous system) or on peripheral pain mechanisms, without significantly altering consciousness[1]. Due to having adverse side effects, like gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as analgesic agents have not been successful in all the cases. Therefore, analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant based drugs (Kumara, 2001)[2].

Kausik Biswas, Ishita Chattopadhyay under review have also shown that different types of extracts from various parts of neem tree (bark, seed, leaf) have analgesic, anti-inflammatory, anti-pyretic, immunostimulant, hypoglycemic, anti-ulcer, anti-fertility, anti-malarial, antibacterial, antifungal, anti-viral, anti-carcinogenic, antioxidant, hepatoprotective effects [3].

More than 135 compounds have been isolated from different parts of neem. Some of them such as nimbin, nimbinin, nimbidin, nimbolide and nimbidic, are biologically active [4]

*Azadirachta indica* (Family: Meliaceae) is a fast-growing tree that can reach a height of 15-20 m, rarely to 35-40 m. Neem (*Azadirachta indica*) commonly called 'Indian Lilac' or 'Margosa', belongs to the family Meliaceae, subfamily Meloideae and tribe Melieae. Neem is the most versatile, multifarious trees of tropics, with immense potential. It possesses maximum useful non-wood products (leaves, bark, flowers, fruits, seed, gum, oil and neem cake) than any other tree species. These non-wood products are known to have antiallergenic, antidermatic, antifeedent, antifungal, anti-inflammatory, antipyorrhoeic, antiscabic, cardiac, diuretic, insecticidal, larvicidal, nematicidal, and spermicidal and other biological activities. Because of these activities neem has found enormous applications making it a green treasure.[5] The main active constituents of the plant are nimbin, nimbinin, nimbidin, limocinol, limocinone, azadirol, naheedin, azadironolide, limbocinin[1]. A literature survey reveals that no systematic approach has been made to study the analgesic activity of fresh juice of young stem bark of *A. indica* A. Juss plant.[6] Plant *Momordica charantia* Linn. belongs to family *Cucurbitaceae*. It is known as bitter gourd in English and *karela* in Hindi. Earlier claims show that the plant is used in stomachic ailments as a carminative tonic; as an antipyretic and antidiabetic agent; and in rheumatoid arthritis and gout[1]

It is cultivated throughout India, Malaya, China, Tropical Africa, and America. Earlier claims showed that its bitter fruits have carminative, aphrodisiac, and anthelmintic properties, and are used in syphilis, rheumatism, troubles of spleen, and ophthalmia. It is also useful in piles, leprosy, jaundice, and also used as a vermifuge. Upon a literature review, it was found that the plant contains moisture (83.2%), proteins (2.9%), fat (1.0%), carbon (9.8%),

fibers (1.7%), mineral matters (1.4%), calcium, phosphorus, iron, carotene, thiamine, nicotinic acid, riboflavin, ascorbic acid (88 mg/100 g), copper, and potassium. Charantin, β-sitosterol-glucoside, stigmast-5, 25-dien-3 β-*O*-glucoside, stigmast-7,25-dien-3 β-ol, and stigmast-7, 22,25-trien-3 β-ol are isolated from the fruit. Many pharmacological properties have been reported including antioxidant, adipogenesis-reducing, antilipolytic, hypoglycemic, antidiabetic, anticancer, antifertility, antigenotoxic, anthelmintic, antimicrobial, antiviral, and hepatoprotective activity. However, there are no reports to our knowledge on its analgesic and antipyretic activities. Hence, the present study was undertaken to investigate the analgesic and antipyretic potential of the ethanolic and aqueous fruit extract of *M. charantia* Linn. in experimental animal models[1].

Leaves are administered internally in leprosy, piles, jaundice. It is active as galactoguge; it is also applied round the eye orbit for night blindness. Leaf juice is rubbed to soles in burning of the feet, and used in liver complaint of children's. In Cambodia and in Gold coast, leaves are also considered to be antipyretic[7]

## MATERIALS AND METHODS

### *Plant Material*

The *Momordica charantia* Linn. And *Azadirachta indica* A. Juss Leaves for the proposed study were collected and they were authenticated by department of botany of RTMU Nagpur University, Nagpur. The freshly collected leaves of *Momordica charantia* Linn. And *Azadirachta indica* A. Juss were shade dried and then powdered to coarse size. About 500 gm of leaves powder of *Momordica charantia* Linn. And *Azadirachta indica* A. Juss was subjected to extraction with (ethanol 95 %). After extraction, the solvent was distilled off and the extracts were concentrated on water bath. Then prepare the formulation and formulations were evaluated for hepatoprotective activity.

Fig.1- Leaves of *Momordica charantia* Linn

Fig.2- Leaves of *Azadirachta indica* A. Juss

### *Preparation of Polyherbal formulation*

The quantity of ethanolic extracts of leaves required for formulating herbal drug formulation (Table 1) are calculated on the basis of human dose of powder form and percentage practical yield of respective crude drugs. Two formulations are prepared using 2% w/v gum tragacanth as suspending agent and considered as Lower dose and higher dose formulation [1.4]

**Table 1**
***Quantity of plant extracts used for preparing herbal formulations F1 and F2***

| Sr. No. | Extract Name | Quantity of Extract mg/kg **(F1)** | Quantity of Extract mg/kg **(F2)** |
|---|---|---|---|
| 1 | *Momardica charantia* Linn | 300 | 500 |
| 2 | *Azadirachta indica* A. Juss | 200 | 500 |

***Test Animal***
The experimental protocol was submitted and approved by Institutional Ethical Committee, Wister albino rats (150-200 g) of approximate same age were employed in this investigation. The animals were fed with standard pellet diet and water and ad libitum. They were housed under standard conditions of temperature $22^0$ C ($\pm$ $3^0$ C) humidity 35 % to 60 %, and light (12:12 hr light/dark cycle) in polypropylene mice cage. The animals received the drug treatments by oral gavages tube.

***Chemicals***
Diclofenac sodium was obtained as a gift sample from Merck, India and the other chemicals and reagents used were of analytical grade.

***Acute toxicity studies***
Acute toxicity studies were carried out on Wister albino rats according to method proposed by Ghosh. The prepared formulation were subjected to toxicity study and were found to be safe up to daily dose of 4000 mg/kg of body wt. in rats of either sex with no toxic reaction being observed.

***Analgesic activity*** [8, 9]
Analgesic activity of Polyherbal formulation i.e. F1 and F2 were studied by eddy's hot plate and heat conduction method.
All the experiments were conducted on an isolated and noiseless condition. The analgesic activity was evaluated by the Eddy's hot plate method and by heat conduction method using Analgesiometer in rats. All Formulations for analgesic activity were administered orally. The standard drug Diclofenac sodium was administered in the form of solution in water for injection as vehicle. For the assessment of analgesic activity in each method the animals of either sex were divided into five groups each composed of six animals. All groups received intraperitoneal injection (maximum 1 ml as per ethical norms).

Group I: Control animals received 5% Tween 80 at the dose of 10 ml/kg. Response) were noted at 0, 30 min, 60 min and 90 min and 120 min. As the reaction time

Group II: Animals received standard Diclofenac sodium at the dose 9mg/kg.

Group III: F1

Group IV: F2

***Heat conduction method***

The animals were divided into five groups of 6 animals each. Group I served as control. Group II served as standard and were injected Diclofenac sodium (9 mg/kg) intraperitoneal. Group III and Group IV were treated orally with formulation F1 and F2 respectively. After one hour, the tip of tail was dipped up to 5 cm into hot water maintained at 58°C. The response time was noted as the sudden withdrawal of the tail from the hot water. Cut off time of 10 seconds was maintained to avoid damage to the tail for all groups. The time required for flicking of the tail, was recorded, to assess response to noxious stimulus [8, 9]

***Eddy's hot plate method***

The animals were divided into five groups of 6 animals each. Group I served as control. Group II served as standard and were injected Diclofenac sodium (9 mg/kg) intraperitoneal. Group III and Group IV were treated orally with formulation F1 and F2 respectively. The animals were individually placed on the hot plate maintained at 55°C, one hour after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. The cut off time for the reaction was 15 seconds. [8.9]

***Statistical analysis***

All the values ware statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey- Kramer multiple comparison test. Comparison between control and drug treated groups were considered to be significant. All values are expressed as mean ± SEM.

## RESULT AND DISCUSSION

**Heat conduction method:** Animals treated with formulation F1 and F2 showed significant increase in the tail flick latency compared to control. The tail flick latency at a F1 formulation (lower dose) was found to be 7.42 sec after 90 min of drug treatment whereas the standard drug diclofinac sodium showed the tail flick latency 8.51 sec (Table-2) (Figure-2). The activity was also found to be a significant activity.

**Table 2**

***Shows analgesic activity of formulations F1 and F2 fresh, by heat conduction method.***

| Treatment | Tail- flick latency in Sec ( Mean ± S.E.M.) at time (min) | | | | |
|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 90 min | 120 min |
| Control | 2.55 ± 0.51 | 2.44± 0.45 | 2.77 ± 0.34 | 2.42 ± 0.39 | 2.52 ± 0.45 |
| Standard | 2.53 ± 0.31 | 4.79 ± 0.78** | 7.65 ± 0.66** | 8.51 ± 0.43** | 7.35 ± 0.76** |
| F1 | 2.45 ± 0.23 | 3.92 ± 0.51** | 5.65 ± 0.55** | 7.42 ± 0.35** | 5.45 ± 0.37** |
| F2 | 2.41 ± 0.23 | 3.52 ± 0.46** | 4.75 ± 0.35** | 6.23 ± 0 .52** | 4.31 ± 0.36** |

*All values are expressed as mean ± S.E.M. (n= 6)*

*** P< 0.01, * P < 0.05 significant compared to control*

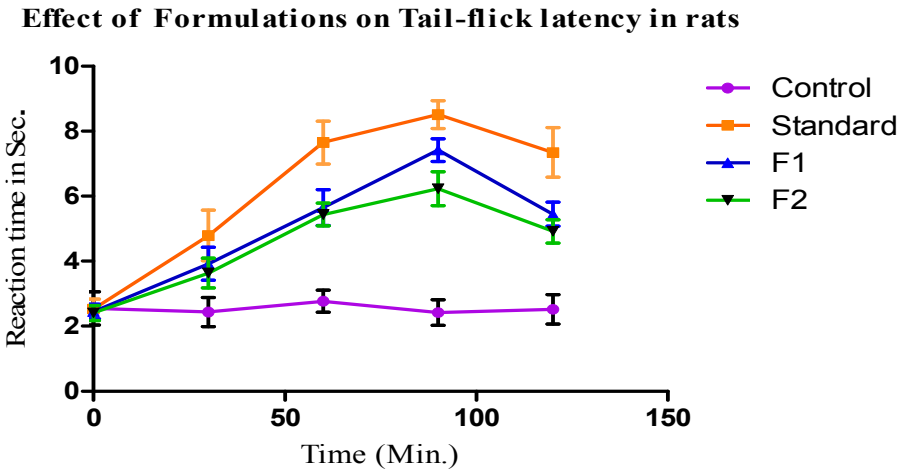


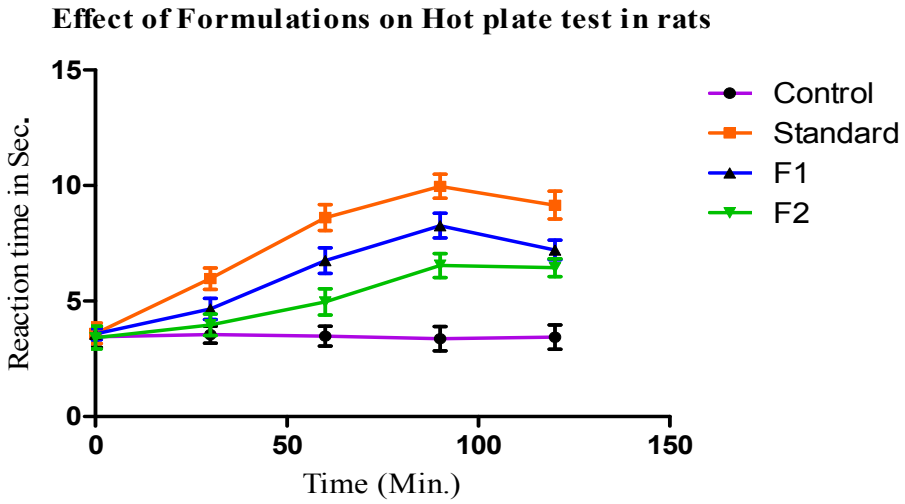

**Figure 2**
***Effect of Formulations on Tail- flick latency in rats***

***Hot plate method***: Animals treated with Formulation F1 and F2 showed significant and dose dependent analgesic activity in thermal stimulated pain (hot plate test) in rats. The reaction time at a formulation F1 (Lower dose) was found to be 8.26 sec after 90 min of drug treatment whereas the standard drug diclofenac sodium showed the tail flick latency 9.96 sec. (Table-3) (Figure-3).

**Table 3**
***Shows analgesic activity of fresh juice of young stem (tender) bark of Azadirachta indica A. Juss. By Eddy's hot plate method***

| Treatment | Reaction time in Sec ( Mean ± S.E.M.) at time (min) | | | | |
|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 90 min | 120 min |
| Control | 3.45 ± 0.46 | 3.54± 0.37 | 3.48 ± 0.43 | 3.37 ± 0.53 | 3.43 ± 0.53 |
| Standard | 3.60 ± 0.44 | 5.97 ± 0.46** | 8.61 ± 0.56** | 9.96 ± 0.52** | 9.15 ± 0.61** |
| F1 | 3.57 ± 0.24 | 4.65 ± 0.46** | 6.74 ± 0.55** | 8.26 ± 0.53** | 7.21 ± 0.42** |
| F2 | 3.41 ± 0.49 | 3.97 ± 0.46** | 4.96 ± 0.57** | 6.54 ± 0 .52** | 6.44 ± 0.39** |

*All values are expressed as mean ± S.E.M. (n= 6)*
*** P< 0.01, * P < 0.05 significant compared to control*




**Figures 3**
***Effect of Formulations on Hot plate test method in rats***

## DISCUSSION

The authenticated and collected plant leaves of *Momordica charantia* Linn. And *Azadirachta indica* A. Juss were shade dried and then powdered to coarse size. About 500 gm of leaves powder of *Momordica charantia* Linn. And *Azadirachta indica* A. Juss was subjected to extraction with (ethanol 95 %). After extraction, the solvent was distilled off and the extracts. The present study explores the Polyherbal formulation for Analgesic activity of ethanolic leaves extracts of *Momardica charantia* Linn and *Azadirachta indica* A. Juss against experimental model of Eddy's hot plate method And Heat conduction method response in rats. Polyherbal suspensions were prepared by the trituration method using a suspending agent and ethanolic dried extract of leaves. The formulation of *Momardica charantia* Linn and *Azadirachta indica* A. Juss were evaluated for analgesic activity. In both of Eddy's hot plate method And Heat conduction method response in rats writhing response method; the intraperitonially administration of formulation F1 and F2 induced a significant analgesic activity in a dose-dependent manner respectively in the rats. Formulation F1 has shown significant analgesic effect than the F2 with compare of Diclofenac sodium as a standard drug. The plant may have the phytoconstituents which inhibit cyclooxygenase enzyme or act on central opioid receptors. Based on the results of the present study, it can be concluded that formulation F1 showed signification analgesic activity than F2 in rats.

## CONCLUSION

In conclusion, we can confirm that the formulation F1 showed the potent analgesic as compared to the formulation F2 in rats.

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