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A STUDY ON ANALGESIC ACTIVITY OF AQUEOUS EXTRACT OF *MORINGA OLEIFERA.L* FLOWERS IN ALBINO RATS

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ABSTRACT

The International Association for the Study of Pain's widely used as definition the pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage". In the medical diagnosis, pain was regarded as a symptom of an underlying condition. In pharmacological screening method, *Moringa oleifera. L* flowers extraction when administered in rats shown less potent analgesic activity when compared to the standard drug, by using Eddy's hot plate method maintained at 55^{o}C temperature. The phytochemical study it was proved that flavanoids, alkaloids, tannins, saponins, phenolic compounds, proteins and carbohydrates are present. From the study it was shown that the Ethanolic extract low and high doses shown more significant response when compare with control.

KEYWORDS

Moringa oleifera, Eddy's hot plate method, Tail flick method and Analgesic activity.

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INTRODUCTION

The International Association for the Study of Pain's widely used as definition pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage. In medical diagnosis, pain was regarded as a symptom of an underlying condition.

Pain motivates the individual to withdraw from damaging situations, to protect a damaged body part while it heals, and to avoid similar experiences in the future (Fernado *et al*, 2012). Most pain resolves

once that the noxious stimulus was removed and the body has healed, but it may persist despite removal of the stimulus and apparent healing of the body. Sometimes pain arises in the absence of any detectable stimulus, damage or disease (Raj *et al*, 2007)[1].

Different types of pain

Acute Pain

Is defined as short-term action but extreme pain that comes on quickly but last only for a brief period of time. This type of pain responds well to medications (Irina AS *et al*, 2003).

Chronic pain

It is defined as pain that persists longer than the normal course of time associated with a particular type of injury. It was more difficult to treat other than acute pain. The experience of physiological pain can be grouped according to the source and related nociceptor (pain detecting neurons) (Charles R Craig *et al*, 2008).

Somatic pain

Its originates was ligaments, tendons, bones, blood vessels etc. It is detected with somatic nociceptors. The scarcity of pain receptors in these areas produces a dull, poorly localized pain of longer duration than cutaneous pain; examples include Sprains and broken bones (Irina AS *et al*, 2003).

Visceral pain

The visceral nociceptors is <1% in comparison with somatic afferents and the cortical mapping of visceral afferents is also less concentrated (Irina AS *et al*, 2003).

Cutaneous pain

Is caused by injury to the skin or superficial tissues. Cutaneous nociceptors terminate just below the skin and due to the high concentration of nerve endings, produce a well-defined, localized pain of short duration (Bjorn AM *et al*, 2006).

Neuropathic pain or neuralgia

Can occur as a result of injury or disease to the nerve tissue itself. This can disrupt the ability of the sensory nerves to transmit correct information to the thalamus and hence the brain interprets painful stimuli even though there is no obvious or known physiologic cause for the pain.

Phantom limb pain

Is the sensation of pain from a limb that has been lost or from which a person no longer receives physical signals. It is an experience almost universally reported by amputees and quadriplegics (Rang HP *et al*, 2003).

Pathways of Pain

Nociception is conveyed from the periphery to the brain by an adaptable and dynamic pathway. The pathway is transmitted and modulated at three levels: the peripheral nociceptor, the spinal (dorsal horn of the cord) and the supraspinal (brain). Peripheral activation most pain originates after tissue damaged. The release of inflammatory mediators from tissues, immune cells and sympathetic and sensory afferent nerve fibers results in an 'inflammatory soup' bathing the nociceptors. These chemicals can directly activate or sensitize the high-threshold nociceptors to activation by low intensity stimuli (Rang HP *et al*, 2003) (Charles R Craig *et al*, 2008).

BIOLOGICAL SOURCE

It consists of dried long, slender, triangular seed pods of *Moringa oleifera. L.*

Geographical source

It is native to sub-Himalayan areas of India, Pakistan, Bangladesh and Afghanistan. It is also grown in the tropical regions.

Cultivation and collection

Moringa oleifera is believed to be native to sub-Himalayan tracts of northern India but is now found worldwide in the tropics and sub-tropics. It grows best in direct sunlight under 500 meters altitude. It tolerates a wide range of soil conditions, but prefers a neutral to slightly acidic (pH. 6.3-7.0), well-drained sandy or loamy soil. Minimum annual rainfall requirements are estimated at 250mm with maximum at over 3,000mm, but in waterlogged soil the roots have a tendency to rot.

CHEMICAL CONSTITUENTS

phytol, 1, 2-benzene dicarboxylic acid, 3, 4-epoxy ethanone, oleic acid, dimethyl phenol, alkaloids,

steroids, flavonoids, tannins, saponins, carbohydrates and minerals.

Uses

- Analgesic
- Arthritis
- Cancer
- Constipation
- Gastritis
- Ulcerative colitis
- Depression

QUALITATIVE ANALYSIS

Preliminary Phytochemical Screening

The collected extracts were subjected for phytochemical screening using freshly prepared reagents to analyze the present phytoconstituents in extracts. The extracts were analyzed for the detection of alkaloids, glycosides, flavonoids, proteins, amino acids, carbohydrates and tannins. (Kokate*et al*, 2017).

For detection of Alkaloids

To the 2g dried extracts, dilute HCl was added, and stirred with glass rod and filtered. The filtrate obtained was subjected to following tests;

Dragendorff's reagent test

Few drops of dragendorff's reagent were added to 2-3ml of filtrate on a watch glass and observed for appearance of orange brown precipitates which reveal the presence of alkaloids.

Mayer's reagent test

Few drops of Mayer reagent were added to 2-3ml of filtrate on a watch glass and observed for appearance of cream coloured precipitates which revealed the presence of alkaloids.

Test with Wagner's reagent test

Few drops of Wagner's reagent were added to 2-3ml of filtrate on a watch glass and observed for appearance of reddish brown precipitates which revealed the presence of alkaloids.

Hager's reagent test

Few drops of Hager's reagent were added to 2-3ml of filtrate of watch glass and observed for appearance of yellow precipitates which revealed the presence of alkaloids.

For detection of phenolic compounds

$Fecl_3$ Solution test

Few drops of ferric chloride solution were added to 2-3ml of the extracts and observed for appearance of deep blue black colour revealing the presence of phenolic compounds.

Lead acetate solution test

Appearance of white precipitate was observed after addition of few drops of lead acetate solution to 2-3ml of extracts. Formation of white coloured precipitates revealed the presence of phenolic compounds.

For detection of Saponins

About 2gm of dried extracts were dissolved in water, filtered and subjected to the following test,

Foam test

The extracts were shaken vigorously with water and observed for the appearance of persistent foam revealing the presence of saponins.

For detection of Carbohydrates

About 2g of dried extracts were dissolved in water, filtered and subjected to following test.

Molisch's reagent test

To 2-3ml of extracts few drops of α-naphthol solution in alcohol were added, shaken and concentrated sulphuric acid was added from sides of test tube and observed for the formation of violet ring at the junction of two layers showing the presence of glycosides and carbohydrates.

Fehling solution test

1ml each of Fehling' solution A and B were mixed and boiled for one minute. Equal volume of test solution was added and heated on a boiling water bath for 5-10 minutes and observed for the appearance of yellow, then brick red precipitates revealing the presence of reducing sugars.

Benedict's reagent test

Equal volume of Benedict's reagent and test solution was mixed in a test tube and heated on boiling water bath for 5 minutes and observed for the appearance of green, yellow and red coloured solution depending on the amount of reducing sugar present in the solution.

Barfoed's test

Equal volume of Barfoed's reagent and test solution was mixed in a test tube and heated on boiling water bath for 1-2 minutes, cooled and observed for the appearance of red coloured precipitates exhibiting the presence of monosaccharides.

For detection of Proteins and Amino Acids

Millon's reagent test

To 3ml of test solution of extract 3 drops of Millon's reagent were added and appearance of white precipitates. The white precipitates were warmed and observed for formation of brick red colour precipitates giving red coloured solution showing the presence of protein in the extract.

Ninhydrin reagent test

To 3 ml of test solution of extract 3 drops of 5% Ninhydrin solution were added, heated on water bath for 10 minutes and observed for appearance of purple or bluish color revealing the presence of amino acids.

For detection of Glycosides

50mg of extract is hydrolysed with concentrated hydrochloric acid for 2h on water bath, filtered and the hydrolysate is subjected to the following tests.

Borntrager's test

To 2ml of filtered hydrolysed, 3ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates the presence of glycosides.

Legal's test

The 50 mg of extract was dissolved in pyridine; sodium nitroprusside solution is added and made alkaline using 10% sodium hydroxide. Presence of glycoside is indicated by pink colour.

For detection of flavonoids

About 2g of dried extracts were dissolved in ethanol, filtered and subjected to following test.

Shinoda test

The dried extracts was dissolved in 95% ethanol (5ml) and few drops of concentrated hydrochloric acid (HCl) were added. Then magnesium turnings were put into the solution and appearance of pink colour indicated the presence of flavonoids.

Lead acetate solution test

Small amount of solutions in lead acetate solution was added and observed for appearance of formation of yellow colored precipitates.

For detection of Steroids

Liebermann-Burchard's test

To 2 ml of extract with chloroform, 1-2 ml of acetic anhydride and 2 drops of concentrated sulphuric acid was added from the sides of test tube and observed for appearance of red, then blue and finally green colour.

For detection of tannins

About 0.5g of extract were added and then add in 10 ml of water in a test tube and filtered. Add 0.1% ferric chloride was added and observed for brownish green or blue-black coloration.

PHARMACOLOGICAL STUDIES

Approval of protocol:

All the experimental procedure and protocols used in the present study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) constituted under Committee for the Purpose of control and supervision of experiments on animals (CPCSEA).

Procurement and Housing of Animals

Animals were purchased from the CPCSEA registered breeder, Raghavendra Enterprises, Bangalore. The animals were housed in an air conditioned room and were kept in standard laboratory conditions under natural light and dark cycle (approximately 12h light / 12h dark cycle) and maintained humidity 60 ± 5% and an ambient temperature of 25 ± 2°C. The animals were allowed to free access to standard diet and water ad libitum and allowed to acclimatize for one week before the experiments. Commercial pellet diet contained 22% Protein, 4% Fat, 4% Fiber, 36% Carbohydrates and 10% Ash w/w, supplied by Raghavendra Enterprises, Bangalore were used.

Eddy's Hot Plate Test

Procedure

The animals were divided into four groups of five animals each. Group I served as control. Group II served as standard and were injected diclofenac

sodium (5mg/kg, ip). Group III and IV were treated orally with AEE of 200 and 400mg/kg body weight, respectively. The animals was individually placed on the hot plate maintained at 55°C, one hour after their respective treatments. The response time were 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[13]. Further to investigate the effect of naloxone on rats along with AEE, animal were divided into two groups of five animals each. After 30 min animals form both groups were subjected for trial on hot plate and change in response was observed.

Tail Flick Test

Procedure

The animals were divided into four groups of five animals each. Group I served as control. Group II served as standard and were injected diclofenac sodium (5mg/kg) intraperitonially. Group III and IV were treated orally with AEE of 200 and 400mg/kg body weight respectively. After one hour, the tip of tail were dipped up to 5cm 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.

Drug treatment

Wistarrat's were divided into four groups of 5 animals each.

Group I - Received Control (2% saline)

Group II - Received Standard drug (diclofenac-5mg/kg i.p)

Group III- Test-Received low dose (200mg/kgp.o)

Group IV - Test-Received high dose (400mg/kgp.o)

Acute toxicity studies[14, 15]

Acute toxicity tests were performed in mice. All animals were fasted overnight before treatment and were given food 1 h after AEMC treatment. A single high dose (400mg/kg), as recommended by the OECD guidelines was administered orally to mice. General behavior was also observed at 1, 3 and 24 h after administration.

RESULTS AND DISCUSSION

Extraction

Size reduced powder of flowers of *Moringa oleifera* were extracted separately by Soxhlet extraction technique with aqueous (70%). Extractive yield from respective solvents.

Percentage yield of the extracts:

The percentage yield of the collected extracts was calculated accordingly and was found as mentioned in Table No.1.

Result of Phytochemical Screening

Result of Preliminary phytochemical screening of aqueous extract of *Moringa oleifera* flowers.

Statistical analysis

Statistical analysis was performed using graph pad prism 6. The Values were expressed as mean ± SEM and one way analysis of variance (ANOVA) was used for statistical analysis.

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Discussion

The medicinal plant *Moringa oleifera. L* is collected from the medicinal plant garden of VISHWA Bharathi College of Pharmaceutical Sciences, Perecherla, Guntur (d.t). This plant authentification was done in department of botany in Sri Venkateswara University.

In order to test the analgesic activity we have taken the reference or standard drug Diclofenac sodium. We selected the 30 rats for control, test and standard samples with regular intervals and taken the wash out period three days for each type of sample. Inject the sample to the rats through intraperitoneal route and oral dose in certain doses at regular intervals of time 0min, 30min, 60min, 90min, 120min and note the basal reaction response (jump response) in seconds by using Eddy's hot plate method at 55c.

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Compare the response of control, standard drug and observe the responses by plotting the time (min) on X-axis and basal reaction response (jump response) in sec on Y-axis.so the standard response was increases compare to control, the standard response had more significant value.

Table No.1: Percentage yield of the collected extracts

S.No	Extract	Weight taken (grams)	Percentage Yield
1	Aqueous extract of *Moringa oleifera*	200	28%

Table No.2: Result of Phytochemical Screening

S.No	Phytochemical	Aqueous extract of *Moringa oleifera*
1	Carbohydrates	+
2	Glycosides	-
3	Flavonoids	+
4	Saponins	**+**
5	Alkaloids	+
6	Proteins and amino acids	+
7	Phenol and phenolic compounds	+
8	Tannins	+

+ indicates-present, - indicates-absent

Table No.3: Eddy's hot plate method

S.No	Groups	Dose (mg/kg)	Basal reaction time(jump response) in sec(avg)				
			0 min	30min	60min	90min	120min
1	Group1-Control	-	0	9±1.32	8±1.33	9±1.38	10±1.39
2	Group II-Standard-Diclofenac sodium	5mg/kg	0	16±1.52	19±1.72	20±1.81	16±1.43
3	Group III-Test-A Aqueous extract-low	200mg/kg	0	11±1.92	14±1.99	18±1.86	12±1.65
4	Group IV-Test-B Aqueous extract-high	400mg/kg	0	14±1.82	17±1.88	19±1.56	12±1.23

Table No.4: Tail-flick method

S.No	GROUPS	Dose (mg/kg)	Flicking responses in sec(avg)				
			0 min	30min	60min	90min	120min
1	Group1-Control	-	0	8±1.32	10±1.22	9±1.52	8±1.92
2	Group II-Standard-Diclofenac sodium	5 mg/kg	0	15±1.52	19±1.12	18±1.62	14±1.82
3	Group III-Test-A Aqueous extract-low	200mg/kg	0	10±1.62	14±1.32	14±1.72	11±1.72
4	Group IV-Test-B Aqueous extract-high	400mg/kg	0	13±1.72	16±1.42	17±1.52	12±1.62

Figure No.1: About plant

Percentage Yield = (Weight of Extracts obtained/Weight of Crude extracts) X 100

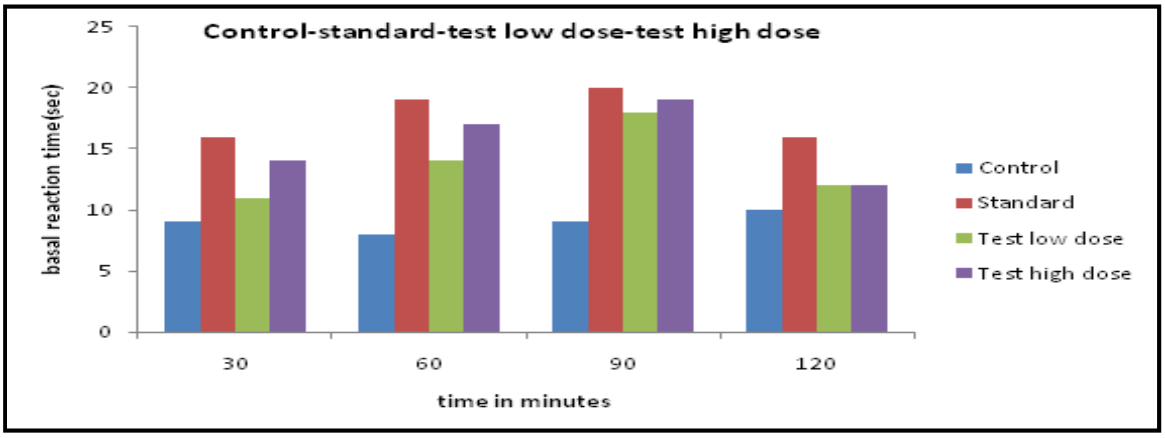

Figure No.2: Eddy's hot plate method

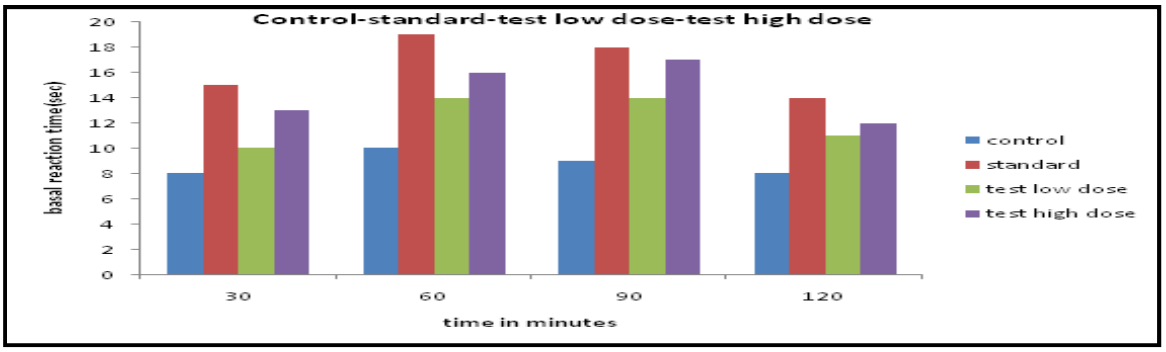

Figure No.3: Tail-flick method

CONCLUSION

In pharmacological screening method, *Moringa oleifera.L* seeds extraction when administered in rats shown less potent analgesic activity when compared to the standard drug, by using Eddy's hot plate method maintained at 55°C temperature and also *Moringa oleifera.L* seeds extraction when administered in rats shown less potent analgesic activity when compared to the standard drug, by using tail flick method.

The phytochemical study it was proved that flavanoids, alkaloids, tannins, saponins, phenolic compounds, proteins and carbohydrates are present.

From the study it was shown that the Ethanolic extract low and high doses shown more significant response when compare with control.

And it was proved that *Moringa oleifera.L* shows fewer side effects than individual.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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