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Research Article

Chemical Investigation and Studies of Analgesic and Antipyretic Activity of Moringa oleifera *lam.* Seeds Extract

Mohammed Rageeb[1], Mohammed Usman[1*] and SD. Barhate[2]

[1]JJT University, Jhunjhunu, Rajasthan, India, 333001.
[2]Shri Suresh Jain Institute of Pharmaceutical Education and Research Center, Jamner, Maharashtra, India.

ABSTRACT

This work has been done for the investigation and study of analgesic and antipyretic activity of *Moringa oleifera* lam. seeds extract. analgesics and antipyretics compounds in the market still present a wide range of undesired effects leaving an open door for new and better compounds. Natural products are believed to be an important source of new chemical substance with potential therapeutic applicability. Qualitative chemical investigation for the identification of chemical constituents. Identification of the active principles by TLC and Column chromatography and further studies them for possible analgesic activity by using Hot plate method and Tail immersion method and antipyretic activity by Yeast induce hyperpyrexia method in Female albino rats. The present study indicates that Hot plate and Tail immersion model tests suggests that the ethanolic extract seems to possess an intensity of analgesic effect that is mostly mediated via a peripheral mechanism by inhibition of the PGs-mediated potential of analgesic action of bradikinin and Yeast induced Hyperpyrexia method showed that the ethanolic extract at dose (30 mg/kg) caused significant lowering of the body temperature up to 2 hr, as the mean temperature 38.28 ºc was reduced to 37.30 ºc. It is thus evident that *Moringa oleifera* Lam. is a weak analgesic and antipyretic agent as compared to Aspirin.

Keywords: *Moringa oleifera* lam., Analgesic activity, Antipyretic activity, Aspirin, Pharmacognostical.

INTRODUCTION

The nature has provided a compete storehouse of remedies to cure all ailments of mankind. Since the dawn of civilization, in addition to food crops, man cultivated herbs for his medicinal needs. The knowledge of drugs has accumulated over thousands of years as a result of man's inquisitive nature, so that today we possess many effective means of ensuring health-care. The human being appears to be affiliated with more diseases than any other animal species. There can be little doubt that, he very early, sought it alleviate his sufferings from injury or disease taking advantage of plants growing around him .In the past ,almost all the medicines used were from plants, the plants being mans only chemist for ages. Today there has accumulated a vast store of knowledge concerning the therapeutics properties of different plants. The opoid analgesics and their derivative have never been surpassed as painkillers in efficacy or patient acceptability despite their disadvantages [(1)]. Phytotherapy offers several approaches which have been shown to be clinically effective and for which biochemical and pharmacological data are available [(2)]. In India, many generations of medical treatment has been recorded in the Ayurveda, one of the earliest collections of Hindu medical lore. The doctrine itself dates back much earlier; to the Rig Veda (references on 67 herbal drugs can be found). Charka Samhita (900 B.C.) is the first recorded treatise on Ayurveda, which describes 341 plants and plant products for use in medicine. Shushruta Samhita (600 B.C.) records many more herbal remedies and even laid special emphasis on surgery Bhava Mishara's Bhava Parkas' (1550 A.D.)

is one of the last celebrated treatise on Hindu system of medicine.

A small or medium-sized tree up to 10 m tall, with thick, soft, corky, deeply fissured bark and tomentose twigs. Seeds are Trigonous, the wings angled. Flowers and fruits once or twice each year, depending on locality; in central India, where trees remain leafless between December-January and January-February, flowering occurs mainly between November and March, and fruiting from February to June. The seed contain a newly developed glycoside moringine[3] ,4(alpha-L Rhamnoloxy)Benzylisothiocynate in seed[4], alanine, arginine, glycine ,serine[5], acidic, stearic, palmitic, linoleic[6]. The seeds are acrid, bitter, anodyne, anti-inflammatory, purgative, antipyretic and ophthalmic. They are useful in neuralgia, inflammations, intermittent fevers and opthalmopathy [7]. The fruit (pod) is used to treat diseases of the liver and spleen, articular pains, tetanus and paralysis [8].

MATERIALS AND METHODS

Procurement and authentication of drugs

The Seeds of *Moringa oleifera* lam were collected from local areas of Chopda Maharashtra and authenticated by Department of Botany, Agharkar Research Institute, pune, The Voucher no. is F-164.

Drying and size reduction

In the present study, 3kg. dried seeds of *Moringa oleifera* lam were reduced to coarse power using mechanical grinder and passed through a sieve No.40 to obtained about 3 kg powder of desired particle size.

Extraction and fractions

About 250 gm powder was subjected to hot continue extraction (soxhlet) with ethyl alcohol (95%) in a soxhlet extractor at a temp of 45^0 to 50^0c to 40 cycles per batch for 12 batches. The extraction was continuing until the solvent in the thimble becomes clear indicating the completion of extraction. After the complete extraction, the solvent was distilled off and concentrated on a water bath to a dry residue. Some part of the total extract was reserved for phytochemical investigation and assessment of analgesic and antipyretic activity.

The concentrated ethanol extract (about 250 g) was dispersed in 250 ml of distilled water and subjected to fractionation by using petroleum ether ($40\text{-}60^0$), solvent ether, ethyl acetate, butanol in succession. Each fraction was washed with water, then dried over anhydrous Na_2So_3 (Sodium Sulphite) and concentrated to a small volume and then evaporated to dryness The dried ethanol extract and its fractions were stored in a desiccators and used for further experiment after suspending in gum acacia 2%. The chemical constituents of the ethanol extract and its fractions were identified by preliminary qualitative analysis and shown in Table 1.

QUANTITATIVE CHEMICAL INVESTIGATION OF EXTRACTS

The alcoholic extract and various fractions were subjected to qualitative chemical investigation for the identification of the active principles [9, 10], Shown in Table 2.

Identification of active principle by Thin Layer Chromatography

For detecting the presence of amino acid

Adsorbent	– Silica gel G (activated)
Plate size	– 20 cm X 8 cm
Plate thickness	– 0.2 mm
Solvent system	– Water: Ethanol: Acetic acid (1: 5:0.1)
Spraying agent	– Ninhydrin solution
Developing time	– At 110^0c for 10 min

The R_f values of alcoholic extract and various fraction of *Moringa oleifera* lam. seeds [11], Shown in Table 3.

Column chromatography of alcoholic extracts

Selection of Mobile phase

The solvent system developed for TLC was used as mobile phase for column chromatography Alteration in the composition of eluting solvent gradually to a reservoir of the first line with efficient mixing.

Preparation of column

The slurry of silica gel G (60-120 mesh size) was prepared by mixing the adsorbent with mobile solvent. Solvent poured into the column. A cotton wool was placed at the base of column and air bubble inside the cotton wool was removed. Small amount of sand poured into column in order to provide flat base .Slurry of adsorbent poured gradually and allow settling. The air trapped was removed by stirring with glass rod. A filter paper disc was placed above the adsorbent. Sand was added. The excess solvent then run off until the level fall to 1 cm above the top layer of sand.

Details of column chromatography

Adsorbent	: Silica gel G for column chromatography activated at 105^0c. For 1 hour
Length of column	: 41cm.
Diameter of column	: Outer 3 cm. and Inner 2.8 cm
Rate of elution	: 10-15 drops/min.
Volume of each Eluent collected	: 25 ml each
Total Volume of Eluent collected	: 100 ml.
Elution	: Chloroform: Acetone and Acetone: Methanol

Totally around 84 Eluent were collected and each eluent was subjected to thin layer chromatography as described above for identification of amino acid. The R_f value has been calculated.

Assessment of analgesic and antipyretic activity

Animal selection

Female albino rats weighting between, 150-200gm were used for acute toxicity study of various extracts. The animals were lasted overnight prior to the experimental products. Albino rats, Wister strain of weighting 150-200gm. were used for analgesic and anti-pyretic models. Rats were kept in polyprolene cages and led on standard laboratory diet and ad libitum. The animals were exposed to 12 hours of darkness and light each. The bedding materials of cages were changed everyday. Rats were divided into group of six.

Acute toxicity study

Acute toxicity study was carried out according to OECD guidelines (organization for economic co-operation and development) [12].

Preparation and administration of doses

The test compound i.e. ethyl alcohol extract, petroleum ether fraction, ethyl acetate fraction, n-butanol fraction was administered orally. Animals are fasted prior to dosing with free access to water. The dose of 1ml/100gm b.w. of all test materials was given to the mice in stepwise procedure using little doses of 5, 50, 300 and 2000mg/kg b.w. food was given to the mice 3 to 4 hr. after administering the test materials. Signs and symptoms of toxicity were observed at 2000 mg/kg in single animal for all extracts in singing study. The same dose was given to three animals for main toxicity study.

Analgesic and antipyretic activity

In this group of 6 albino rats of both sexes with a weight between 150-200gm are used for each dose. Eight groups are made each containing 6 animals.

Group I	: Test compound
Group II	: Standard (Aspirin)
Group III	: Ethyl alcohol extract

Group IV : Petroleum ether extract
Group V : Solvent ether
Group VI : Ethyl acetate extract
Group VII : Butanol extract

Analgesic activity
Hot plate method
In hot plate method group of 10 mice of either sex with an initial weight 18 to 22g are used for each dose. The hot plate, which is commercially available, consists of a electrically heated surface. The temperature is controlled for 55^0 to 56^0C. This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stopwatch. The latency is recorded before and after 20.60 and 90 min following oral or sub-coetaneous administration of the standard or the test compound [13], Shown in Table 4 and Figure 1.

Tail immersion method
Young female Wister rat are used. They are divided into 8 group having 6 animals are allowed to adapt to the cages for 30 min. before testing. The lower 5 cm. Portion of the tail was marked. This part of the tail is immersed in a cup of freshly filled water of exactly 55^0C. Within a few second the rats reacts by withdrawing the tail. The reaction time is recorded in 0.5s. Units by a stop watch. After each determination the tail is carefully dried. The reaction time is determined before and periodically after either oral or subcutaneous administration of a substance. The cut off time of the immersion is 15s withdrawal time of untreated animals is between 1 and 5.5s.A withdrawal time of more than 6 s therefore is regarded as a positive response [14], Shown in Table 5 and Figure 2.

Antipyretic activity
Yeast induce hyperpyrexia method
By insertion of a thermocouple to a depth of 2cm into the rectum the initial rectal temperature are recorded. The animals are fevered by injection of 10 mg/kg of brewer's yeast suspension subcutaneously in the back below the hope of the neck. The sight of injection is massaged in order to spread the suspmical. The room temperature is kept at 22-24'c. Immediately after yeast administration. Food is withdrawn 18 h. post challenge, the rise in rectal temp. of at least 38'c are taken into the test. The animals react the test compound or standard drug by oral administration. Rectal temperature is recorded again 30, 60,120, and 180 min. post dosing [15], Shown in Table 6 and Figure 3.

RESULTS AND DISCUSSION

Phytochemical Investigation
The result of qualitative chemical investigation of 5 fractions has indicated the presence of following compounds.

Ethanol : Sterols, Carbohydrates, Alkaloids, Glycoside, Amino acid, Flavonoids and Proteins.
Petroleum ether (40-60) : Sterols, Carbohydrates, Amino acids, Fats
Solvent ether : Carbohydrates, Proteins, Fats, oil.
Ethyl acetate : Sterols, Carbohydrates, Tannins and Alkaloid.
Butanol : Carbohydrates and Proteins

Presence of amino acid in ethanolic extracts was identified by TLC profile. The extract show two spot on TLC with R_f value 0.86 (pink) and 0.96 (violet) in solvent system water: ethanol; acetic acid (1:5:0.5) solvent system. The elution obtained from ethanolic extract by column chromatography were showed single spot on TLC profile with R_f value 0.86 (pink) in Water: Ethanol; Acetic acid (1: 5:0.5) solvent system.

Pharmacological screening

Acute Toxicity Study

Acute toxicity study was carried out according to OECD (Organization of Economic Cooperation and Development) guideline in albino rats. The acute toxicity study of various extracts of *Moringa oleifera* Lam. seeds was showed signs of toxicity like tremor, convulsion and deep breathing at 300mg/kg b.w. 1/10th of the same dose for all these exacts were taken as therapeutic dose i.e. 30mg/kg.b.w.

Analgesic model

The analgesic activity of various extracts of *Moringa oleifera* Lam. was assessed by Hot plate model and tail immersion model. The results obtained from the Hot plate model and Tail immersion model indicates that ethanolic extract of treated group showed (P<0.001) reduction in pain from 1 hr onwards when compared to control group.

The results of analgesic activity of the above two methods have been showed in Table 4, 5 and Figure 1, 2.

Antipyretic model

The antipyretic activity of various extracts of *Moringa oleifera* Lam. was assessed by The results Yeast induced Hyperpyrexia method obtained from the Yeast induced Hyperpyrexia method indicates that ethanolic extract of treated group showed (P<0.001) reduction in fever from 2 hr onwards when compared to control group.

The results of antipyretic activity of the above method have been showed in Table 6 and Figure 3.

Table 1: Showing the percentage yield of alcoholics extract and various fractions of seed of *Moringa oleifera* lam

Fractions and Fractions	Weight	% Yield
Ethanol	300.0 gm	10.00
Petroleum ether (40/60)	32.00 gm	1.056
Solvent ether	14.30 gm	0.47
Ethyl Acetate	20.00 gm	0.66
Butanol	41.18 gm	1.35

Table 2: Showing the qualitative chemical investigation of alcoholic extract and various fraction of *Moringa oleifera* lam. seed

Tests and Extract / Fraction	Ethanol Extract.	Pet. Ether (40-60) Fraction	Solvent-ether Fraction	Ethyl acetate Fraction	Butanol Fraction
Test for Sterols	+	+	-	-	-
Salkowski's test	+	+	-	-	-
Sulphur test	+	+	-	-	-
Liebermann Burchards's test	+	+	-	-	-
Test for Glycoside					
Baljet's test	+	-	-	-	-
Keller- Kiliani test	+	-	-	-	-
Raymond's test	+	-	-	-	+
Bromine water test	+	-	-	-	-
Legal's test	+	-	-	-	+
Test for Saponins	-	-	-	-	-
Foam Test	-	-	-	-	-
Haemolysis test					
Test for carbohydrates					-
Molish's Test	+	+	+	+	-
Barfoed's test	-	+	-	-	
Benedict's test	+	-	-	-	-
Test for Alkaloids					
Mayer's test	+	-	-	+	-
Wagner's test	+	-	-	-	-

Dragendorff's test	+	-	-	-	+
Test for Flavonoids					
Ferric Chloride test	+	-	-	-	-
Shinoda test	+	-	-	+	-
Lead Acetate test.	+	+	-	-	-
Test for Tannins					
Ferric Chloride test	-	-	-	+	-
Gelatin test.	-	-	-	-	-
Test for Proteins					
Million's test	+	-	-	-	-
Xanthoprotein test.	+	-	+	-	-

(+) Present (-) Absent

Table 3: Showing the R_f values of alcoholic extract and various fraction of *Moringa oleifera* lam. seed

R_f Value	Colur
0.87	Violet
0.96	Pink

Table 4: Showing the isolation of active principle of alcoholic extract of *Moringa oleifera* seed

Solvents	Concentration	No. of Eluent	No. of Spot	Color of Spot	Avg. R_f Value
Pet. Ether	100	1 to 4	No spot	-	-
Pet. Ether :Benzene	80 : 20	5 to 8	No spot	-	-
	60 : 40	9 to 12	No spot	-	-
	40 : 60	13 to 16	No spot	-	-
	20 : 80	17 to 20	No spot	-	-
Benzene	100	21 to 24	No spot	-	-
Benzene : chloroform	80 : 20	25 to 28	No spot	-	-
	60 : 40	29 to 32	No spot	-	-
	40 : 60	33 to 36	No spot	-	-
	20 : 80	37 to 40	No spot	-	-
Chloroform	100	41 to 44	No spot		
Chloroform : acetone	80 :20	45 to 48	2	Blue violet	0.96 0.82
	60 : 40	49 to 52	2	Blue violet	0.96 0.82
	40 : 60	53 to 56	2	Blue violet	0.96 0.82
	20 : 80	57 to 60	2	Blue violet	0.96 0.79
Acetone	100	61 to 64	1	Blue	0.96
Acetone : methanol	80 : 20	65 to 68	1	Pink	0.87
	60 ; 40	69 to 72	1	Pink	0.87
	40 : 60	73 to 76	1	Pink	0.87
	20 : 80	77 to 80	1	Pink	0.87
Methanol	100	81 to 84	1	Pink	0.87

Table 5: Showing the effect of *Moringa oleifera* lam. seeds extract and fraction on hot plate method

Time in min	Vehicle Control	Std Aspirin 25mg/Kg %Analgesia	Alc. Ext 30mg/Kg %Analgesia	P.E Ext 100mg/Kg %Analgesia	D.E. Ext 300mg/Kg %Analgesia	E.A. Ext 300mg/Kg %Analgesia	B.E. Ext 300mg/Kg %Analgesia
O min	2.00± 0.1581	2.2± 0.3536 (1.10%)	2.80± 0.1581 (4.44%)	2.80± 1581 (4.44%)	2.400± 0.4596 (2.2290%)	2.80± 0.2236 (4.44%)	2.80± 1.089 (4.44%)

15min	2.25±. 1156	6.40± 0.6325 (23.38%)	6.80± 0.3536 (25.63%)	6.80± 0.50 (25.63%)	4.30± 0.6042 (11.54%)	5.00± 1.231 (15.49%)	6.20± 0.7071 (22.25%)
30 min	1.50± 0.07	7.60± 0.7403 (32.9%)	7.50± 0.2236 (32.43%)	6.50± 0.6083 (27.02%)	8.00± 0.5523 (35.13%)	5.30± 1.253 (20.54%)	8.20± 0.7185 (36.21%)
45 min	1.50± 0.2236	10.9± 1.1632 (50.8%)	8.00± 0.6325 (35.13%)	7.85± 0.6103 (34.32%)	7.85± 0.7018 (34.32%)	5.40± 1.077 (21.08%)	7.80± 1.414 (34.05%)

Table 6: Showing the effect of *Moringa oleifera* lam. seeds extract and fraction on tail immersion method

Group	Dose mg/kg	Pretreatment (Sec)	Post treatment (Sec)	Inhibition (%)
Control	-----	2.20±0.1458	1.80±0.2490	-----
Aspirin 25mg/kg	25mg / kg	2.00±0.2850	6.60±0.4123	57.50%
Alcoholic Ext 30mg/kg	30 mg / kg	2.10±0.06083	6.57±0.2909	56.58%
Pet Ether Ext 100mg/kg	100 mg/ kg	2.12±0.6582	6.00±1.024	49.23%
Diethyl Ether Ext 300mg/kg	300 mg/kg	2.08±0.5447	5.80±0.2550	46.96%
Ethyl acetate Ext300mg/kg	300 mg/kg	2.15±0.4962	5.65±o.2871	44.58%
Butanol Ext 300mg/kg	300 mg/kg	2.18±0.09055	5.50±0.1105	46.29%

Table 7: Showing the effect of *Moringa oleifera* lam. Seeds extract and fraction on yeast induced pyrexia method

Group	Rectal Temp °C		Time after medication in min			
	Initial	18 hr after Yeast injection	30 min	60min	90min	120min
Control	38.30 ±0.03162	39.35 ±0.02550	39.30 ±0.01581	39.23 ±0.02550	39.20 ±0.003162	39.15 ±0.03808
Aspirin 150 mg/kg	38.30 ±0.07382	39.35 ±0.07714	38.37 ±0.02550	38.09 ±0.01517	37.67 ±0.05385	37.58 ±0.05831
Alcoholic 30mg/kg	38.28 ±0.02236	39.37 ±0.03808	38.43 ±0.02236	37.87 ±0.06083	37.43 ±0.04472	37.30 ±0.03808
Pet ether 100mg/kg	38.25 ±0.05831	39.39 ±0.08062	38.68 ±0.05148	38.00 ±0.06530	37.88 ±0.05701	37.70 ±0.07489
D.E.300mg/kg	38.27 ±0.06519	39.40 ±0.01275	38.80 ±0.1768	38.60 ±0.1142	38.40 ±0.09618	38.05 ±0.1037
E.A. 300 mg/kg	38.24 ±0.08062	39.38 ±0.1095	38.67 ±0.1300	38.50 ±0.06964	38.40 ±0.08631	38.35 ±0.08019
Butanol 300mg/kg	38.26 ±0.06519	39.40 ±0.05148	38.75 ±0.1118	38.61 ±0.06042	38.58 ±0.06519	38.40 ±0.05263

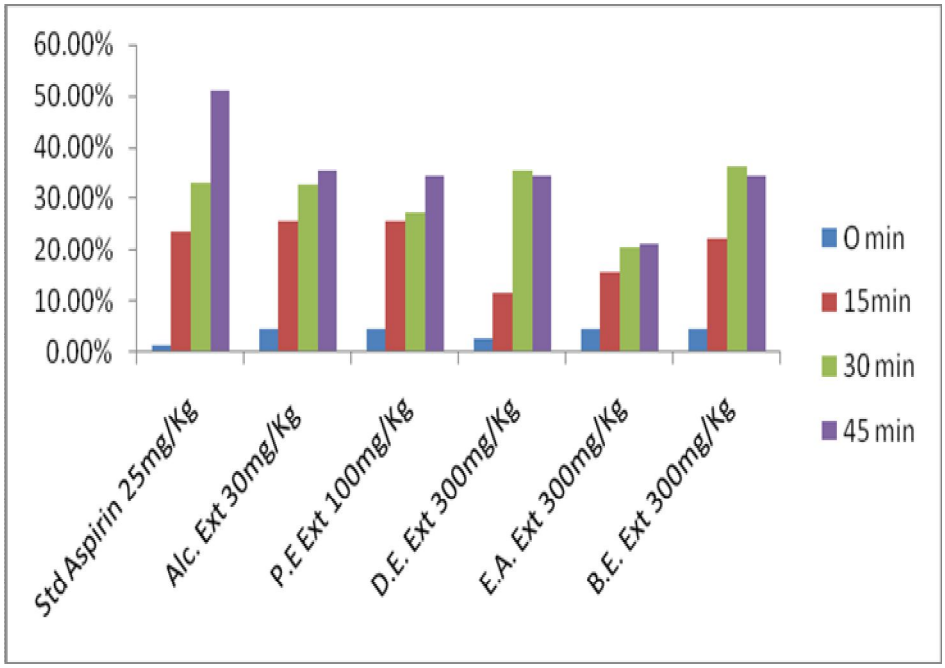

Fig. 1: Analgesic activity of *Moringa oleifera* lam. Seeds extracts and fraction by using Hot plate method

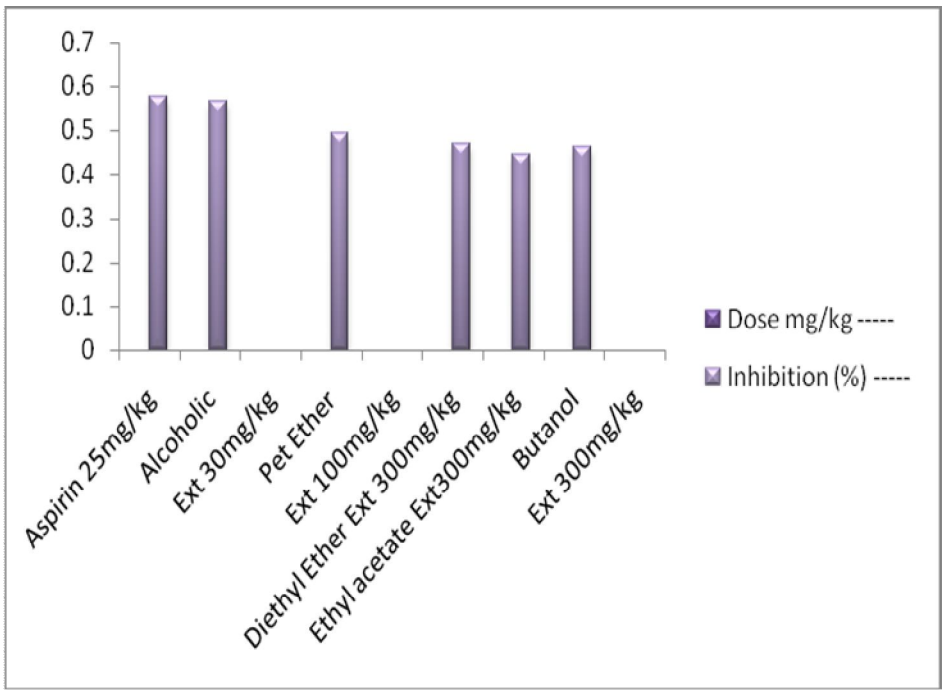

Fig. 2: Analgesic activity of *Moringa oleifera* lam. Seeds extracts and fraction by using Tail immersion method

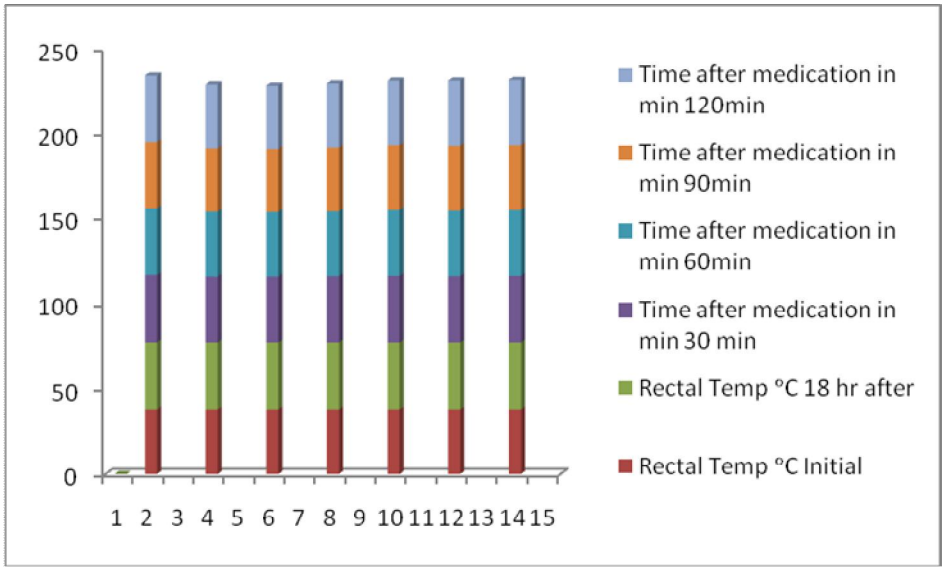

Fig. 3: Antipyretic activity of *Moringa oleifera* lam. Seeds extracts and fraction by using yeast induced pyrexia method

CONCLUSION

Moringa oleifera lam. is a traditionally used herbal drug**.** It has been reported to show anti-inflammatory, purgative, opthalmopathy [(8)]. The presence of amino acid and 4(alpha rhamnosyloxy benzyl isothiocynate) is also reported in seeds [(5)]. In present study morphine a centrally acting analgesic drug produced analgesic effect more than extract in hot plate and in second phase of formalin test. Therefore the potency of extract lowers than morphine. Pretreatment of animal with opoid receptor antagonist, nalaxone decreased the analgesic effect of extract. The result obtained indicates that extract posses a moderate dose dependent analgesic effect on various pain model used. The extract also had a significant effect in various acute pain models, namely tail immersion. Centrally acting analgesic drugs elevate pain threshold of animals towards heat and pressure. The effects of the extract on this pain model indicate that it might be centrally acting. Pyrexia or fever is caused as a secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states.

It was found that maximum inhibitory effect (40.55%) of pain was seen after administration of ethanolic extract (30mg/kg) which was less as compared to standard drug Aspirin (65.55 %) in hot plate model. It was also found that maximum inhibitory effect (56.58%) of pain was seen after administration of ethanolic extract (30 mg/kg) which was more as compared to standard drug Aspirin (57.50%) in Tail immersion model.

The results obtained from Hot plate and Tail immersion model tests suggests that the ethanolic extract seems to posses an intensity of analgesic effect that is mostly mediated via a peripheral mechanism by inhibition of the PGs-mediated potential of analgesic action of bradikinin.

The results of Yeast induced Hyperpyrexia method showed that the ethanolic extract at dose (30 mg/kg) caused significant lowering of the body temperature up to 2 hr, as the mean temperature 38.28 ^{o}c was reduced to 37.30 ^{o}c.

However, the animals did not show any signs of toxicity on administration of *Moringa oleifera* Lam. This indicates that *Moringa oleifera* Lam. is safe in the doses used. It is thus evident that *Moringa oleifera* Lam. is a weak analgesic and antipyretic agent as compared to Aspirin.

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