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Elucidation of anti-arthritic potential of *Morinda pubescens* J.E. Smith var

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Abstract

The Anti-arthritic of methanol extract of *Morinda pubescens* j.e smith var in the dose of 200mg/kg and 400mg/kg respectively.

Method: Wister albino rats were selected, weighed and marked. Test extracts and standard drugs were given to the animal before 30 minutes of administration of formaldehyde. Injected 0.1ml of 2% solution of formaldehyde at the paw of rat on day 1 and 3. Repeated the test or standard drugs for 10 days and measured the paw.

Result: It concludes that the both concentration of *Morinda pubescens* j.e smith var was elucidated and provide significant effect of anti-arthritic activity.

Keywords: arthritic, methanol, morinda pubescens j.e smith var, formaldehyde

Introduction

The pharmacopoeias of many countries of the world include even today a large number of drugs of plant origin ^[1]. Crude drugs of vegetable origin utilized for treatment of disease state often of chronic nature or to obtain or to maintain a condition of good health. Since ancient times, mankind all over the world mainly depended upon plant kingdom to meet all their needs of medicines ^[2].

Medicinal plants still play an important role in emerging and developing countries of Asia, both in preventive and curative treatments, despite advances in modern western medicine ^[2].

There are about 100 types of arthritis which are identified. A joint is the junction where two bones get connected (there may be movement or not). Arthritis mainly affects the joints where inflammation occurs ^[3].

Each joint of the body is attached through synovial joint. When the body immune system starts attacking the synovium, the chemicals released will cause swelling and pain of the joints ^[4]. Osteoarthritis is a degenerative arthritis that involves abnormalities of bones ^[5]. Mechanical injury, overweight and poor muscle strength may be the reason for osteoarthritis ^[6].

Rheumatoid arthritis is a chronic systemic inflammatory disorder that affects the synovial joints. It is the most painful condition. It can also occur at lungs, heart and sclera. The cause of rheumatoid arthritis is unknown. NSAIDs, Disease modifying anti rheumatic drugs and biologicals are the treatment of choice ^[7]. The present study was undertaken to evaluate thearthritis activity of leaves of *Morinda pubescens* J.E. Smith var.in suitable animal model

Materials and Methods

Taxonomical Identification

The plant species was authenticated by Dr. V. Ganesan, Associate Professor and Head Department of Botany, Ayya Nadar Janakiammal College of Arts and Science, Sivakasi, Tamil Nadu, India. The plant species was identified as, *Morinda pubescens* j.e smith var, (MP) Family: Rubiaceae.

Extraction Method

The leaves were collected, shadow dried and then size reduced to small particles manually. The dried material was coarsely powdered before extraction. The extracts were prepared by Soxhlet continuous extraction process by using hexane, chloroform, ethyl acetate and methanol as the solvents. For each solvent, extraction was continued for 48 hours. The colour and percentage yield of each extracts were calculated ^[8].

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Animal

Wistar albino rats weighing 100-150gm were selected. The rats were maintained in a controlled room temperature ($25\pm 2^{\circ}\text{C}$) on 12 hr light/dark cycle with free access to sterile food and water ad libitum [9]. The studies were conducted in accordance with the ethical committee (SBCP/2013-2014/CPCSEA/IAEC-I/3(i)).

Statistical analysis

Values were expressed as mean \pm SEM. The mean difference in body weight changes, paw volume difference, biochemical parameters and haematological parameters were analysed using One way ANOVA followed by Dunnett's test. The values were considered significant at $P < 0.01$ and $P < 0.05$. Analysis was performed using Graph Pad prism statistical software (Version 5.03).

Pharmacological Screening

Formaldehyde induced arthritis

Group	Drug and dose
Group 1	Normal saline (5ml/kg, p.o)
Group 2	Formaldehyde (0.1ml of 2% Intradermally)+Normal saline
Group 3	Formaldehyde (0.1ml+Diclofenac 10mg/kg,p.o.)
Group 4	Formaldehyde + Methanol extra of (200mg/kg,p.o)
Group 5	Formaldehyde + Methanol extract (400mg/kg,p.o)

Formaldehyde is the substance which when injected at the paw of rat will produce arthritis. Wister albino rats were selected, weighed and marked. Test extracts, standard drugs or normal saline were given to the animal before 30 minutes of administration of formaldehyde. Injected 0.1ml of 2% solution of formaldehyde at the paw of rat on day 1 and 3. Repeated the test or standard drugs for 10 days and measured the paw [10].

Results and Discussion

Identification of phytoconstituents

The presence of various phytoconstituents was identified by using different test. Hexane extract found to contain glycosides, coumarin, phenols and xanthoprotein. Chloroform extract was found to contain glycosides, coumarin, steroids, triterpenoids, flavonoids, carbohydrates and xanthoprotein. Ethyl acetate extract was found to contain glycosides, phenols, steroids and triterpenoids. Methanol extract was found to contain alkaloids, carbohydrates, glycosides, phenols, steroids, saponins, terpenoids, coumarins and flavonoids Table 1.

Formaldehyde induced arthritis

In normal control group, the body weight increased normally from 147.5gm on initial day to 172.5gm on final day (day 10). Negative control group showed decrease body weight from 156.25gm on initial day and 142.5gm on day 10th. Standard drug treated group showed i.e., 120gm on initial day and 142.5gm on day 10. of methanolic extract of *Morinda pubescens* J.E. Smith var. (MEMP) 200 treated group increase

in body weight i.e., 125gm on initial day and 152.5gm on day 10. MEMP 400 treated group showed significant increase as in standard i.e., gm on initial day 157.2gm and 200 gm on day 10. So standard drug ($p < 0.01$) and MEMP treated groups ($p < 0.01$) was also found to increase in body weight when compared with the negative control group. The result of mean body weight was shown in Table 2.

The mean body weight change from initial day to day 10th was 25gm for normal animals, 13.75gm for negative control group, 22.5gm for standard treated group, 27.5gm for MEMP 200 treated group and 42.5gm for MEMP 400 treated group. In standard ($p < 0.01$) and MEMP treated group ($p < 0.01$), the body weight change took place normally when compared with the negative control group. The result of mean change in body weight was shown in Table 2.

Effect on paw volume

The mean difference in paw volume was found to be zero in normal control group. Paw volume difference was very much higher for the negative control group i.e., 0.34 on day 10. The group treated with standard drug was found to be with the 0.05 as the mean change in paw volume. The groups treated with MEMP 200 and MEMP 400 were with 0.07 and 0.06 mean change respectively. So when compared with the negative control group, the groups treated with standard ($p < 0.01$) and MEMP was found to be decreased significantly ($p < 0.01$) the paw volume. The results obtained after the estimation of difference in paw volume was shown in the Table 3.

The serum SGOT, SGPT, alkaline phosphate, total protein and creatinine was estimated for formaldehyde induced arthritis. These parameters are very important in determining the disease state. Elevated SGOT, SGPT, alkaline phosphate and creatinine indicated the inflammation state. Decreased total protein level also indicated the same. These factors estimated denote the arthritic inflammation. MEMP treated group decreased the elevated serum levels showing the anti arthritic potential of the methanol extract. The result was shown in the Table 4.

Anaemia is the most common indication in arthritic patients. In patients with rheumatoid arthritis, RBC and Haemoglobin will be decreased and WBC level and ESR will be elevated. WBC was increased as there was an inflammatory condition. These all showed the arthritic condition. Negative control group denoted the severe anaemic condition and the standard and MEMP treated groups were found to be quickly cured. MEMP 200 was also capable of curing the arthritic state. But the potential is very less compared with MEMP 400. The result was shown in the Table 4.

Histopathological evidences proved that in the standard and MEMP treated groups, the inflammation on the joints was very mild when compared with the negative control. All the above discussions proved that the MEMP 200 and MEMP 400 extract have the anti-arthritic potential. But MEMP 400 was less potent than MEMP 200. The result was shown in the Figure 1.

Table 1: Phytochemical screening of *Morinda pubescens* var leaves extracts

Test	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous
Alkaloids	--	--	--	+	--
Glycosides	+	+	+	+	--
Catechin	--	--	--	--	--
Coumarin	+	+	--	+	--

Flavonoids	--	--	--	+	--
Phenols	+	+	+	+	+
Quinones	--	+	--	--	--
Saponins	--	--	--	+	--
Steroids	--	+	+	+	--
Tannins	--	+	--	--	--
Terpenoids	--	+	+	+	--
Xanthoprotein	+	+	+	+	+
carbohydrates	--	+	--	+	--

(+) Presence, (-) Absence

Table 2: Effect of *Morinda pubescens* J.E. Smith var on the mean body weight and mean difference in body weight in formaldehyde induced arthritis in rats

Group	Drug and dosage form	Initial mean body weight	Final mean body weight	Mean difference in body weight
Normal control	Normal saline (5ml/kg,p.o.)	147.5±11.81	172.5±13.14	25±2.9
Negative control	Formaldehyde +Normal saline	156.25±16.75	142.5±19.31	13.75±5.54
Standard	Formaldehyde + iclofenac (10mg/kg, p.o.)	120±10.80	142.5±16.00	22.5±6.3
MEMP	Formaldehyde + MEMP 200mg/kg,p.o.	125±18.92	152.5±23.6	27.5±4.8
MEMP	Formaldehyde + MEMP 400mg/kg,p.o.	157.5±20.56	200±26.14***	42.5±6.30

n=6, Data expressed as Mean± SEM. One way ANOVA followed by Dunnett’s test All groups are compared with Negative control, ***p<0.05, *p<0.01

Table 3: Effect of *Morinda pubescens* J.E. Smith var on mean paw volume change in formaldehyde induced arthritis in rats

Drug and dosage	Mean change in paw volume									
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Normal saline (5ml/kg,p.o.)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Formaldehyde(0.1ml 2% on rat foot ntradermal)	0.23±0.02	0.24±0.02	0.27±0.01	0.29±0.02	0.30±0.03	0.33±0.01	0.35±0.02	0.038±0.03	0.39±0.03	0.41±0.02
Formaldehyde + Diclofenac (10mg/kg,p.o.)	0.27±0.06**	0.26±0.07**	0.25±0.06***	0.21±0.05**	0.20±0.05**	0.17±0.04***	0.14±0.06***	0.10±0.05***	0.07±0.03***	0.03±0.04***
Formaldehyde + MEMP (200mg/kg,p.o.)	0.28±0.06**	0.28±0.06**	0.26±0.04***	0.24±0.05***	0.21±0.07***	0.18±0.06**	0.15±0.04**	0.13±0.03***	0.10±0.05***	0.07±0.02***
Formaldehyde + MEMP (400mg/kg,p.o.)	0.31±0.05*	0.30±0.04**	0.28±0.05**	0.24±0.02**	0.21±0.05***	0.17±0.03*	0.14±0.04**	0.10±0.05**	0.08±0.02*	0.06±0.04**

n=6, Data expressed as Mean± SEM, One way ANOVA followed by Dunnett’s test, All groups are compared with Negative control, ***P<0.05, **P<0.01

Table 4: Effect of *Morinda pubescens* J.E. Smith var on the biochemical parameters in Formaldehyde induced arthritis in rats

Groups	Drug and dosage	SGOT (U/L)	SGPT (U/L)	Alkaline Phosphatase(U/L)	Total Protein (g/dl)	Creatinine(mg/dl)
Normal control	Normal saline (5ml/kg, p.o.)	56±0.91	23.75±0.85	70.50±0.64	7.2±0.27	0.74±0.03
Negative control	Formaldehyde+ Normal saline	96.5±0.85	51.5±2.53	140±1.77	4.76±0.08	1.05±0.01
Standard	Formaldehyde+ Diclofenac (10mg/kg,p.o.)	72±0.46***	32.9±0.73***	80.2±0.21***	5.46±0.85	0.86±0.02***
MEMP	Formaldehyde+ MEMP (200mg/kg,p.o.)	69.91±0.75**	31.83±0.23***	80.25±0.32***	5.52±0.03**	0.78±0.03***
MEMP	Formaldehyde+ MEMP (400mg/kg,p.o.)	67.42±0.52***	29.83±0.70***	76.06±0.37***	6.51±0.21*	0.81±0.008**

n=6, Data expressed as Mean± SEM, One way ANOVA followed by Dunnett’s test, All groups are compared with Negative control, ***P<0.05, **P<0.01

Table 5: Effect of *Morinda pubescens* J.E. Smith var on haematological parameters in Formaldehyde induced arthritis in rats

Drug and dosage	RBC (x10 ⁶ /mm ³)	WBC (x 10 ³ /mm ³)	Haemoglobin (G%)	ESR (mm/hr)
Normal saline (5ml/kg, p.o.)	9.04±0.10	10.11±0.71	12.52±0.20	1.64±0.02
Formaldehyde+ Normal saline	5.81±0.20	14.17±0.42	8.2±0.78	6.1±0.36
Formaldehyde+ Diclofenac (10mg/kg, p.o.)	8.14±0.06***	7.36±0.083***	12.97±0.45***	2.17±0.08***
Formaldehyde+ MEMP (200mg/kg, p.o.)	8.00±0.21***	9.90±0.43***	12.1±0.18***	2.65±0.13***
Formaldehyde+ MEMP (400mg/kg, p.o.)	7.61±0.21***	9.23±0.63***	12.36±0.12***	2.67±0.31***

n=6, Data expressed as Mean± SEM, One way ANOVA followed by Dunnett’s test. All groups are compared with Negative control, ***p<0.05, *p<0.01

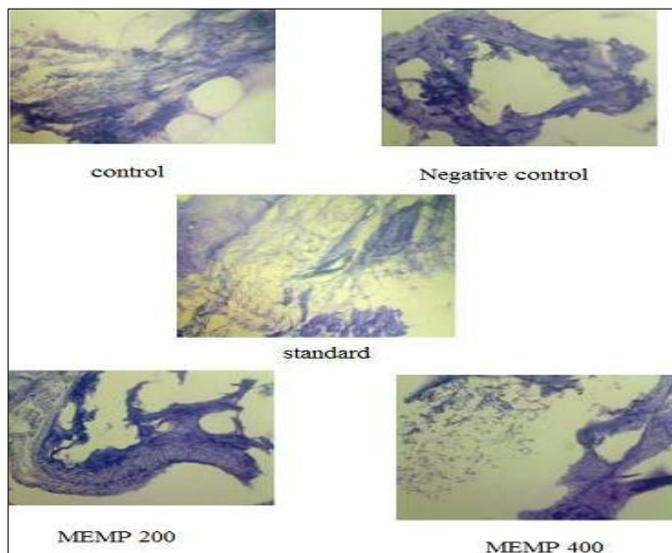


Fig 1: Histopathology of Formaldehyde induced arthritic paw joint tissue

Conclusion

The normal anti arthritic drugs, this *Morinda pubescens* J.E. Smith var extract will not produce any liver and kidney damage. After evaluating the activity of both MEMP 200 mg/kg and MEMP 400mg/kg, MEMP 200 was found to be more active than MEMP400. This result concludes that even though both MEMP 200 and MEMP 400 of the plant contain all the three constituents suspected to be the constituents that possess the anti-arthritic activity. The further works on the plant is on progress at The Department of Pharmacology, Sankaralingam Bhuvanawari College of Pharmacy, Sivakasi

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